NEUROGENETIC TESTING: A CASE-BASED APPROACH

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Ms. Smith has no disclosures.

Clinical Implications of Genetic Testing

- 17 vignettes with questions
- Focus on understanding
  - Uses of genetic testing
  - Test results and mutation nomenclature
  - Common test methods
  - Testing strategy when
    - More than one test method is available
    - More than one gene is associated with a phenotype
    - Testing at-risk relatives
  - Commonly used and abused genetic terms

Three Questions

- Why am I testing this patient at this time?
- What is my plan if the test result is interpreted as “positive”, “negative”, “uninformative”?
- Of the test methods available which is most likely to inform my question about this patient?

Who can help me?

- Why am I testing this patient at this time?
- What is my plan once I receive the test result?

Answer: Neurogeneticist

Medical geneticist

Genetic counselor

Who can help me?

- Which test method is most likely to inform my question about this patient?

- What if I don't understand the interpretation of the test results?

Answer: Laboratory director
Laboratory genetic counselor
Laboratory clinical consultant

What is my plan if the test result is interpreted as “positive”, “negative”, “indeterminate”?

You routinely answer these questions for tests that you order in your area of expertise: MRI, cranial CT, EMG, NCV, etc.

Neurogenetic Testing

- Not “just a blood test”
- Affects entire families
- Significant burden of untreatable disease
- Can predict disease before symptoms
- Issues can vary depending on reason for genetic testing
- Focus on testing for single-gene disorders

Common Concerns

- “Could this information be used against me?”
- Cost and insurance coverage
- Confidentiality of results
- Implications for family members
- Ignorance is bliss?

Reasons for Genetic Testing: Medical Model

- **Symptomatic** - for diagnosis and treatment
  - for refining differential diagnosis
  - for eliminating the need for additional expensive tests
  - for defining natural history and prognosis
- **Presymptomatic/At-Risk** - for surveillance and early treatment options life and family planning purposes. Not done in children under age of 18

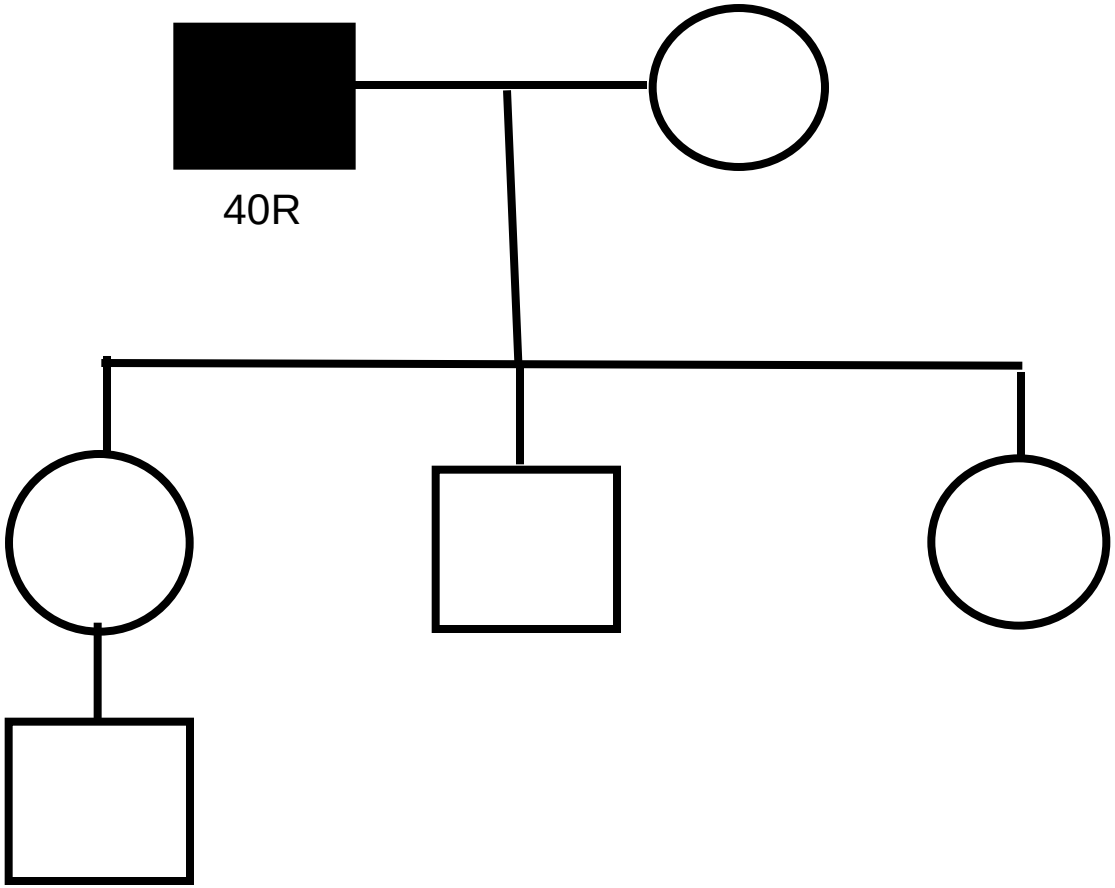
Reasons for Genetic Testing: Personal Decision Model

- **Determining Recurrence Risks**
- **Prenatal** – to prevent recurrence
- **Preimplantation Genetic Diagnosis** – to prevent recurrence without termination
- **Planning for the Future**
- **Obtaining Benefits**
- **Disease Specific Support Organizations**

Case Vignette 1

- Three siblings all in their 30's are at 50% risk to inherit Huntington disease from their father.

Huntington Disease



Case Vignette 1

- Three siblings all in their 30's are at 50% risk to inherit Huntington disease from their father.
- All three siblings request genetic testing

- You would test them

1. Together as a group
2. Individually (one at a time)

Case Vignette 1

- Three siblings all in their 30's are at 50% risk to inherit Huntington disease from their father.
- All three siblings request genetic testing

- You would test them

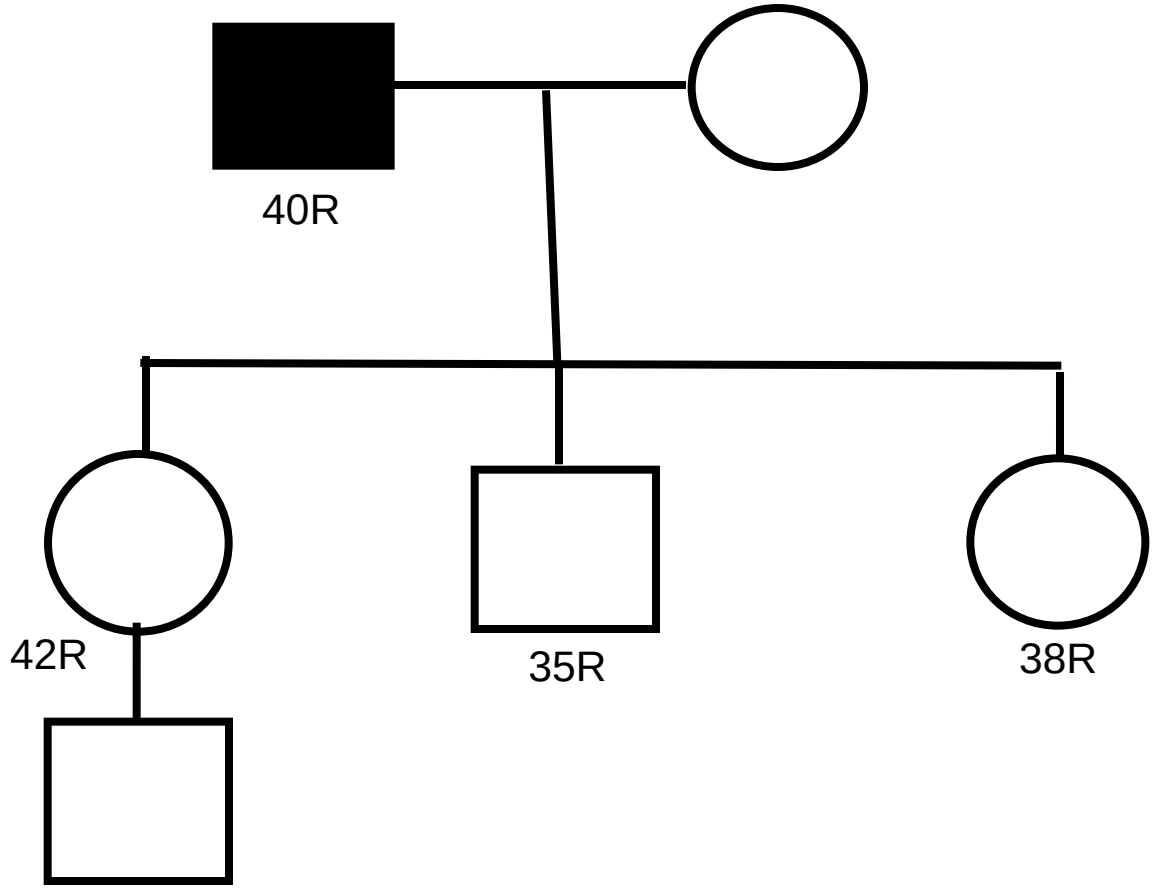
1. Together as a group
2. Individually (one at a time)*

* They may request you modify this plan

Case Vignette 1

- All 3 siblings have DNA testing of the *HD* gene.
  - One has **42** CAG repeats
  - One has **38** CAG repeats
  - One has **35** CAG repeats

Huntington Disease



Case 1: Question

Do the siblings have different risks for developing Huntington Disease?

1. Yes

2. No

Case 1: Question

Do the siblings have different risks for developing Huntington Disease?

1. Yes

2. No

Case 1: Question

Which of the following is true for the sister with 42 CAG repeats?

1. She will eventually develop HD.
2. She will not develop HD, but her children are at risk.
3. She may or may not develop HD.

Case 1: Question

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1. She will eventually develop HD.
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NCBI

GeneTests

Welcome to GeneTests at NCBI

The GeneTests database and Web site are now hosted at NCBI.

We'd like your feedback!

02/15/2011

527 *GeneReviews*
1189 Clinics
597 Laboratories testing for
2271 Diseases
2005 Clinical
266 Research

Laboratory Directory Growth Chart

Administrative Use

(To update Clinic / Laboratory Directory listings)

Welcome to GeneTests

Welcome to the GeneTests Web site, a publicly funded medical genetics information resource developed for physicians, other healthcare providers, and researchers, available at no cost to all interested persons. Use of this Web site assumes acceptance of the terms of use.

At This Site

GeneReviews
Expert-authored peer-reviewed disease descriptions

Laboratory Directory
International directory of genetic testing laboratories

Clinic Directory
International directory of genetics and prenatal diagnosis clinics

Educational Materials
Illustrated glossary, information on genetic services, PowerPoint® presentations, annotated Internet resources

What's New?

New Features

- **Changes to the Management of Laboratory and Clinic Information Online**
- ***GeneReviews* Indexed in PubMed**

New in *GeneReviews*

New Clinical Test Listings

- **9 new listings**

Looking for **Genetic Tools** curriculum materials?

GeneTests is a supplement to and not a substitute for medical advice. Patients with specific questions about genetic counseling or testing should contact their healthcare provider or a genetics clinic.

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We comply with the HONcode standard for trustworthy health information: verify here.

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Trinucleotide Repeat Diseases

Disease	Mode of Inheritance	Trinucleotide Repeat
Huntington disease	AD	CAG
Myotonic dystrophy 1	AD	CTG
Spinocerebellar ataxia 1 (SCA1)	AD	CAG
Dentatorubral-Pallidoluysian atrophy (DRPLA)	AD	CAG
Fragile X syndrome	XL	CGG
Oculopharyngeal muscular dystrophy	AD and AR	GCG
Friedreich ataxia	AR	GAA

Trinucleotide repeat: Sequences of three nucleotides repeated a number of times in tandem within a gene. Normal polymorphic variation in repeat number with no clinical significance commonly occurs between individuals. Abnormally large alleles are classified in increasing order of size as mutable normal alleles, reduced penetrance alleles, and full penetrance alleles, respectively.

Learn More

The sequence of an allele containing four **CAG** repeats looks like this:

A trinucleotide repeat with nine **CCG** repeats looks like this:

Diagnosis

Molecular Genetic Testing

Allele sizes

Full-penetrance HD-causing alleles: **40 or more** CAG repeats. Alleles of this size are associated with development of HD with great certainty.

Case 1: Question

Which of the following is true for the sister with **38** CAG repeats?

1. She will eventually develop HD.
2. She will not develop HD, but her children are at risk.
3. She may or may not develop HD.

Case 1: Question

Which of the following is true for the sister with **38** CAG repeats?

1. She will eventually develop HD.
2. She will not develop HD, but her children are at risk.
3. She may or may not develop HD.

Diagnosis

Molecular Genetic Testing

Allele sizes

Reduced-penetrance HD-causing alleles: **36-39** CAG repeats. An individual with an allele in this range is at risk for HD but may not develop symptoms. In rare cases, elderly asymptomatic individuals have been found with CAG repeats in this range.

Case 1: Question

Which of the following is true for the brother with 35 CAG repeats?

1. He will eventually develop HD.
2. He will not develop HD, but his children are at risk.
3. He may or may not develop HD.

Case 1: Question

Which of the following is true for the brother with 35 CAG repeats?

1. He will eventually develop HD.
2. He will not develop HD, but his children are at risk.
3. He may or may not develop HD.

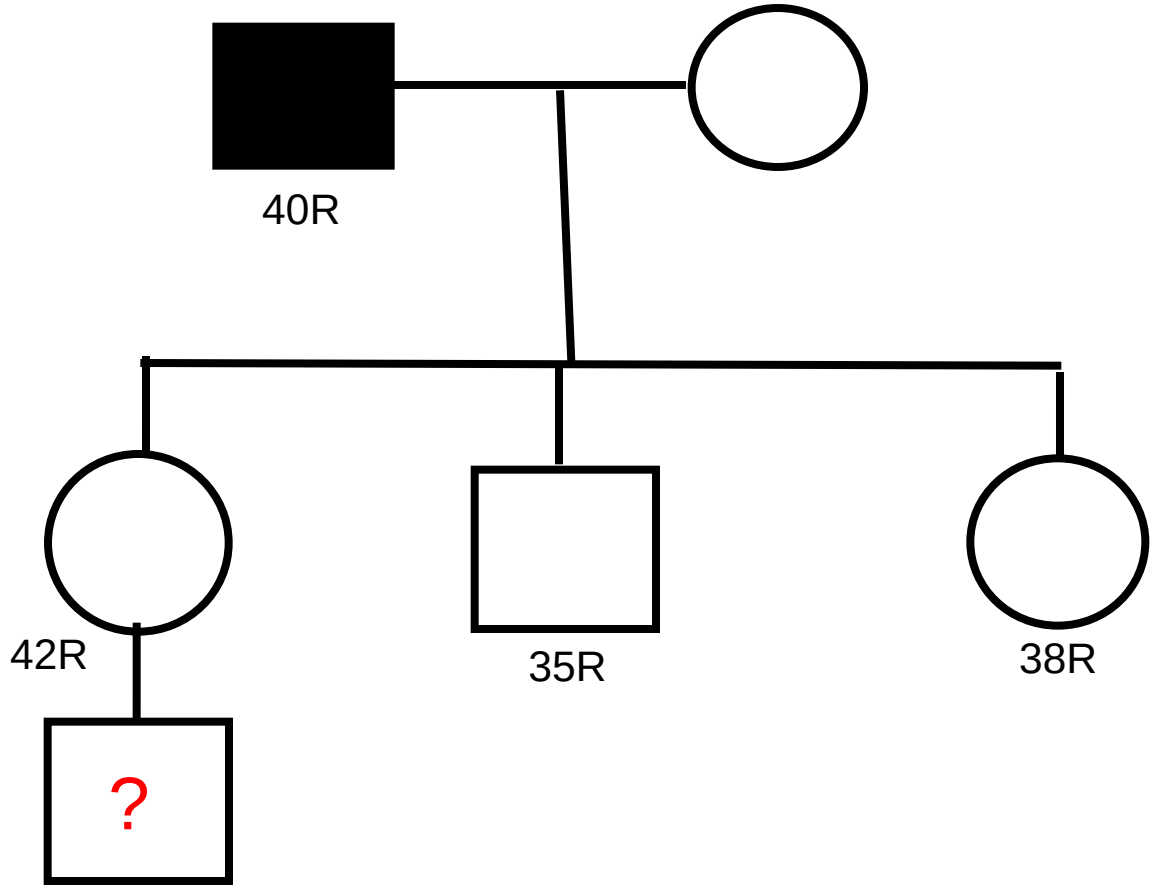
Diagnosis

Molecular Genetic Testing

Allele sizes

Intermediate alleles: **27-35** CAG repeats. An individual with an allele in this range is not at risk of developing symptoms of HD, but because of instability in the CAG tract, may be at risk of having a child with an allele in the HD-causing range. Alleles in the intermediate range have also been described as “mutable alleles”.

Huntington Disease



Case 1: Question

The daughter with 42 CAG repeats requests genetic testing on her 10yo son.

Would you test him?

1. Yes
2. No
3. Maybe

Case 1: Question

The daughter with 42 CAG repeats requests genetic testing on her 10yo son.

Would you test him?

1. Yes
2. No
3. Maybe

Case 1

Predictive Testing:

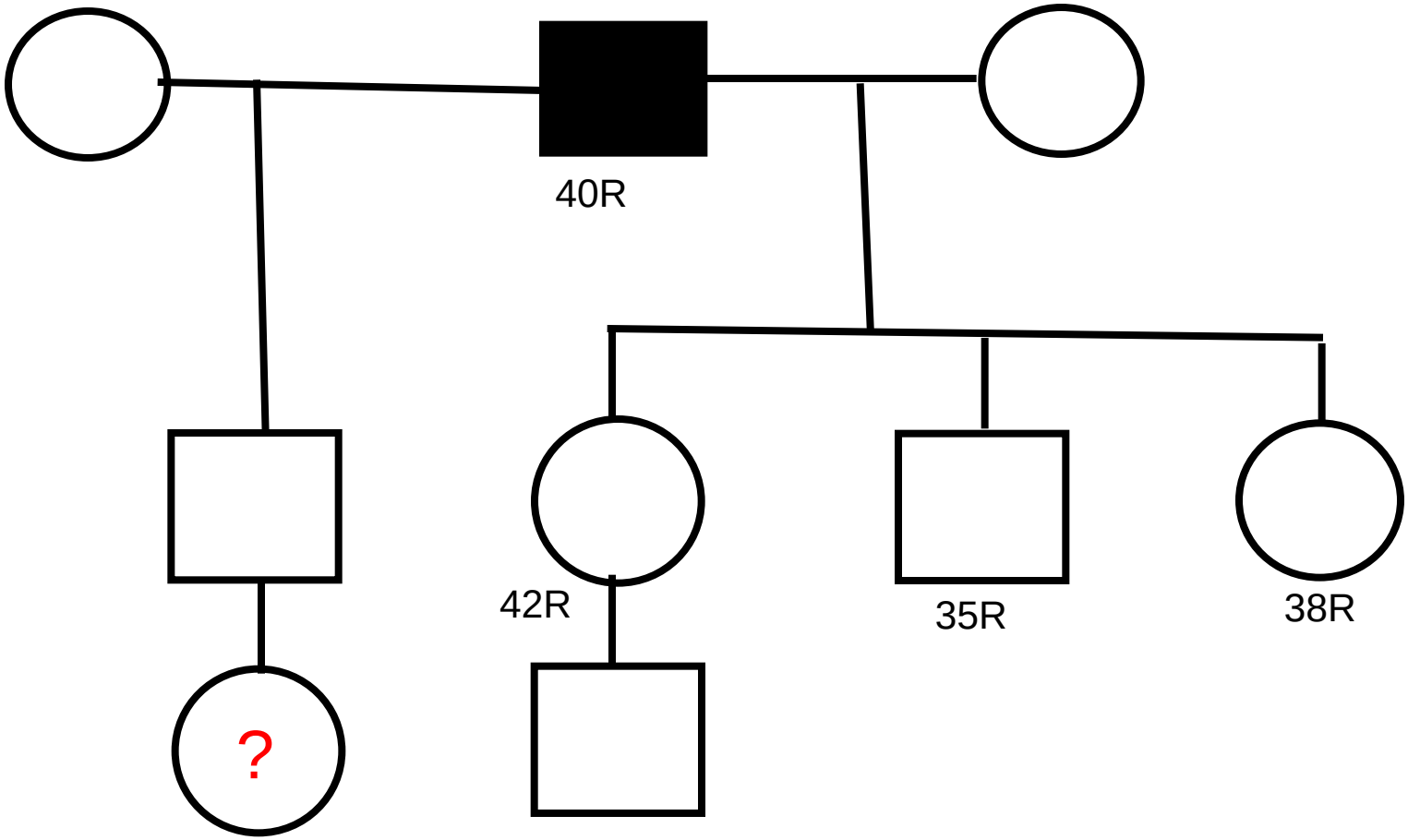
(Syllabus)

- Predictive testing of asymptomatic children at risk for adult-onset disorders is strongly discouraged when no medical intervention is available.
- Exception: Child has symptoms or signs of the disease.

Case 1

- It is discovered that by a previous marriage the father with HD has a son and granddaughter.
- The son refuses testing.
- The granddaughter requests testing.

Huntington Disease



Case 1

- The granddaughter's risk for HD is

1. 50%
2. 10%
3. 25%
4. Cannot determine

Case 1

- The granddaughter's risk for HD is

1. 50%
2. 10%
3. 25%
4. Cannot determine

Case 1

- Would you test the granddaughter?

1. Yes
2. No
3. Maybe

Case 1

- Would you test the granddaughter?

1. Yes
2. No
3. Maybe

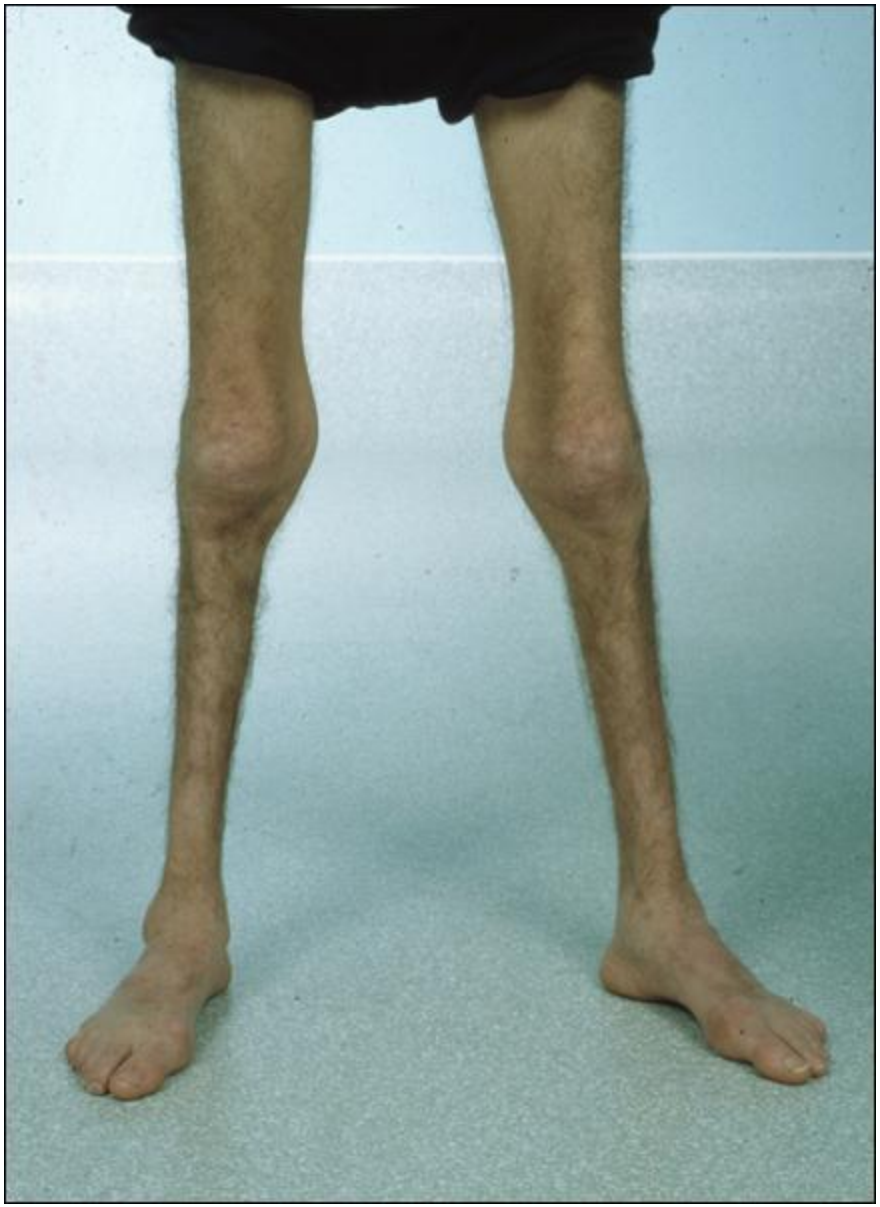
Focus: Case 1

Test results

- Allele sizes in trinucleotide repeat disorders vary by disease.
- The **GeneTests** Web site is a resource for test result interpretation.
- Repeat size may influence phenotype.

Uses of genetic testing

- Presymptomatic testing
- Predictive testing in children
- 25% Risk



Case Vignette 2

A 45 year old man has had a slowly progressive, symmetrical, peripheral neuropathy for 20 years.

- NCV are slow.
- He has no family history of neuropathy.
- His two sons and one daughter are young adults.

Case 2: Questions

Which of the following is the most likely cause of this patient's neuropathy?

1. Autosomal dominant inheritance
2. Autosomal recessive inheritance
3. X-linked inheritance
4. Not genetic
5. Cannot determine inheritance pattern at present

Case 2: Questions

Which of the following is the most likely cause of this patient's neuropathy?

1. Autosomal dominant inheritance
2. Autosomal recessive inheritance
3. X-linked inheritance
4. Not genetic
5. Cannot determine inheritance pattern at present

Case 2: Question

Should this man have DNA testing for CMT?

1. Yes

2. No

Case 2: Question

Should this man have DNA testing for CMT?

1. Yes

2. No

Case 2: Question

Which of the following tests for CMT should be ordered?

1. *PMP22* duplication testing
2. Myelin P zero (*MPZ*) sequencing
3. Connexin 32 (*GJB1*) sequencing
4. All of the above batched together
5. All of the above sequentially

Case 2: Question

Which of the following tests for CMT should be ordered?

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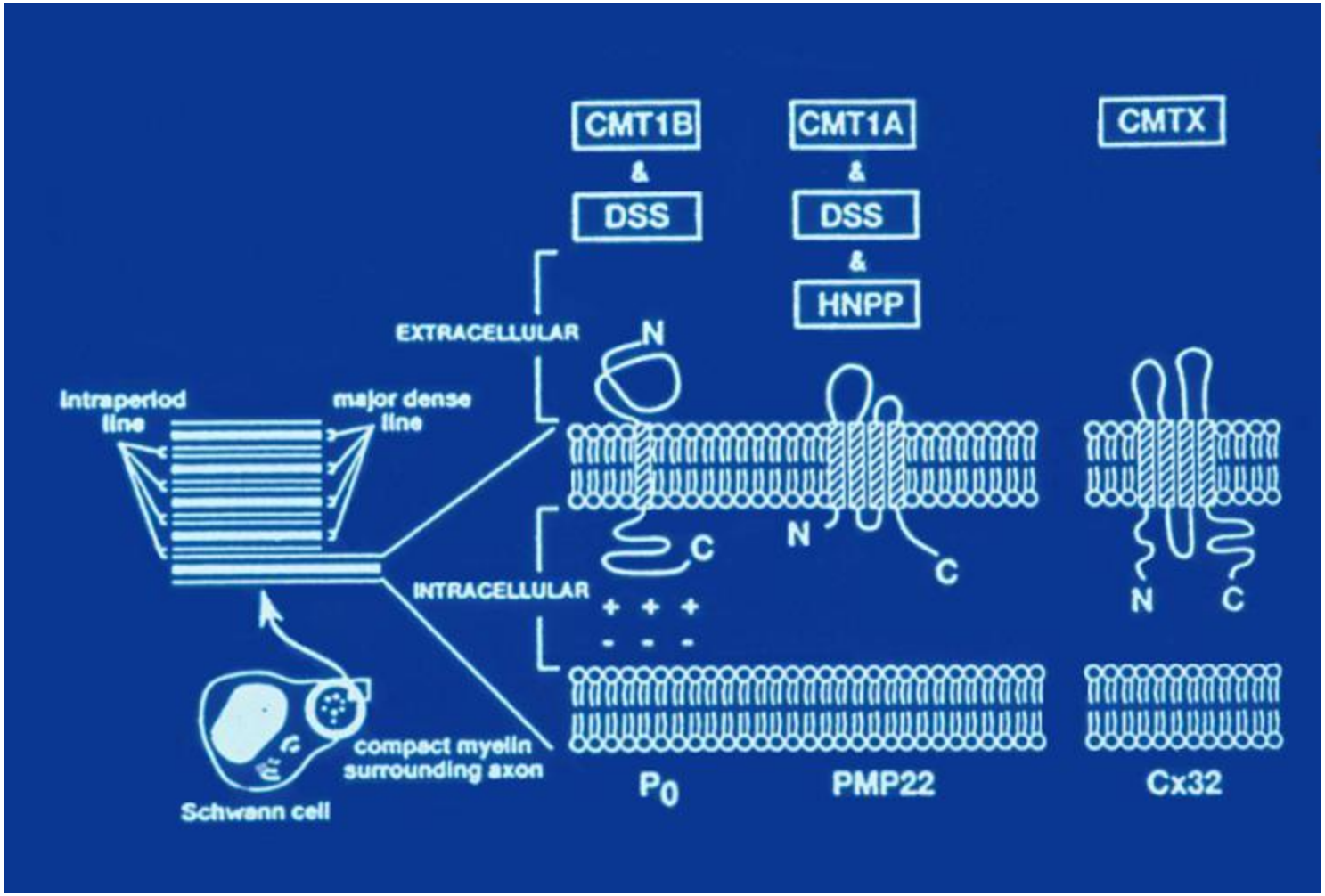

CMT1B
&
DSS
CMT1A
&
DSS
&
HNPP
CMTX
EXTRACELLULAR
N
Intraperiod line
major dense line
C
N
C
N
C
INTRACELLULAR
+ + +
- - -
compact myelin surrounding axon
Schwann cell
P0
PMP22
Cx32

Case 2

Possible testing strategies

- MD: Single sequential testing
- Lab: Batching
- Lab: Tiered approach

Issues to consider

- Subtype prevalence
- Time
- Cost

Prevalence of CMT Subtypes

Strategy	Gene	Proportion of all CMT	Cost	Time
Single sequential	*PMP22* (CMT1A)	~60-70%	$1,070	1 month
	MPZ (CMT1B)	~5%	$965	1 month
	Connexin 32 (CMTX)	~10%	$2,085	1 month
Batched	All 3	~ 75-85%	$4,120	1 month

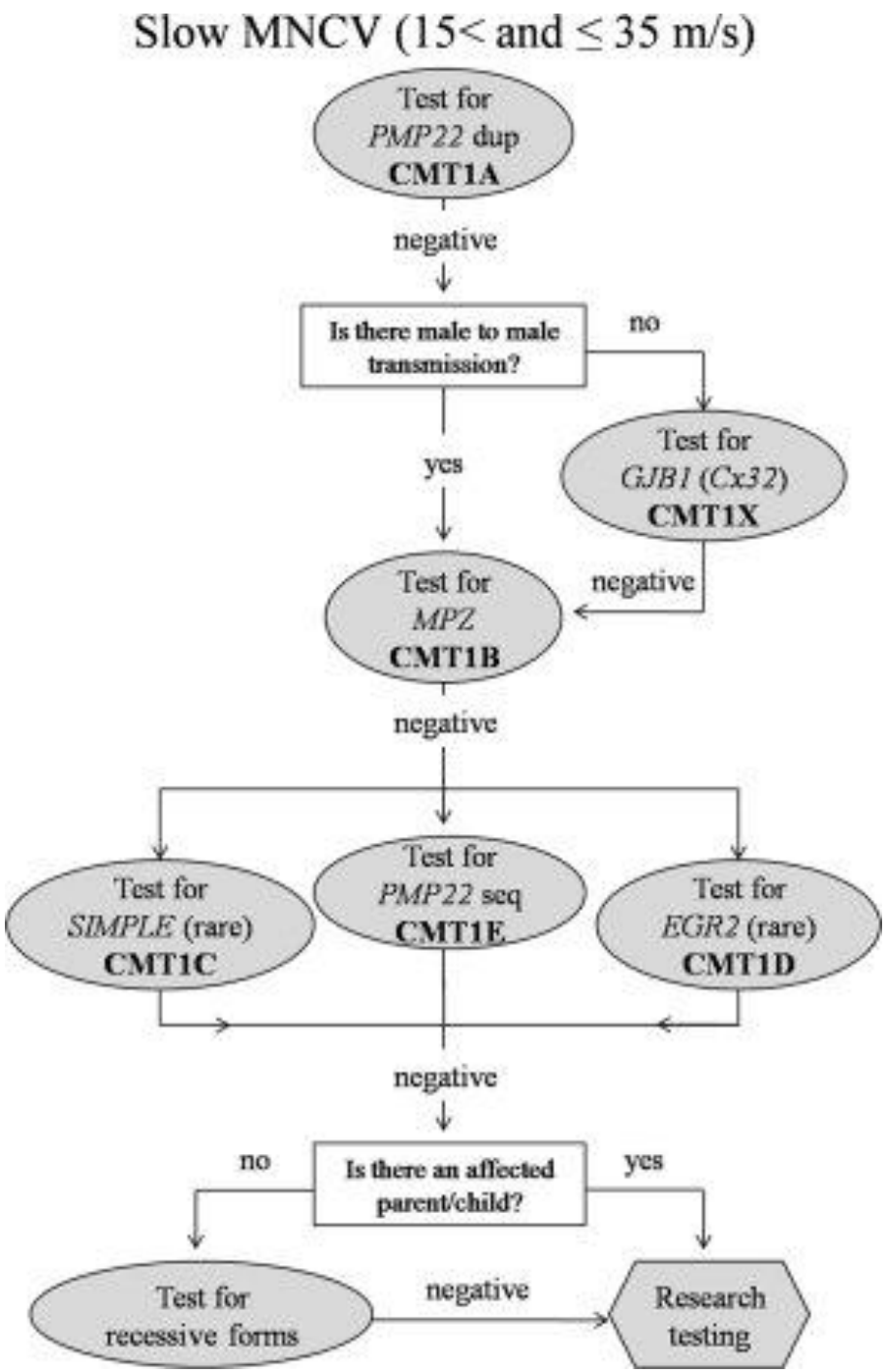
Cost of Batched CMT Tests

CMT Type	# of Tests	Cost
Complete	17	$17,160
Dominant	12	$11,055
Recessive	6	$7,640
Demyelinating	9	$6,810
Axonal	10	$9,490

Charcot-Marie-Tooth Disease Subtypes and Genetic Testing Strategies

Saporta ASD, Sottile SL, Miller LJ, Feely SME, Siskind CE, Shy ME

Ann Neurol 2011; 69: 22-33



Saporta et al 2011

Case 2: Test Results

- The patient has a mutation in *GJB1,* the gene encoding connexin 32. The mutation is T>C at nucleotide 535 (c.535T>C), which results in the amino acid substitution p.Cys179Arg

- This mutation confirms the diagnosis of CMT X

GJB1 Gene

Mutation = p.C179R

Codon 179			Amino Acid
T*	G	C	Cys (C)
↓			
C	G	C	Arg (R)

*Nucleotide 535

Mutation Nomenclature

-Mutations can be named based on different reference sequences

- Different ways of naming the same mutation:

c.535T>C Prefix “c.”=coding reference sequence;
nt #1 = first nt of the first amino acid

p.C179R Prefix “p.”=protein reference sequence;
number = amino acid residue of the protein

g.661T>C Prefix “g.”=genomic reference sequence

Precise mutation nomenclature is important

Knowing precise mutation and gene is essential for future testing in the same or different laboratory for:

- Geographically dispersed relatives
- At-risk relatives upon reaching age 18 yrs
- Prenatal diagnosis
- Preimplantation genetic diagnosis (PGD)

Test reports with precise mutation nomenclature are important

- Check that your institution does not shorten a laboratory report and fail to enter the precise mutation in the electronic medical record of your patient.
- Give the patient of a copy of the test report.

Case 2: Question

What are the risks of CMT X to his children?

1. No risk
2. 50% risk to each child
3. 25% risk to each child
4. No risk to sons / All daughters are carriers

Case 2: Question

What are the risks of CMT X to his children?

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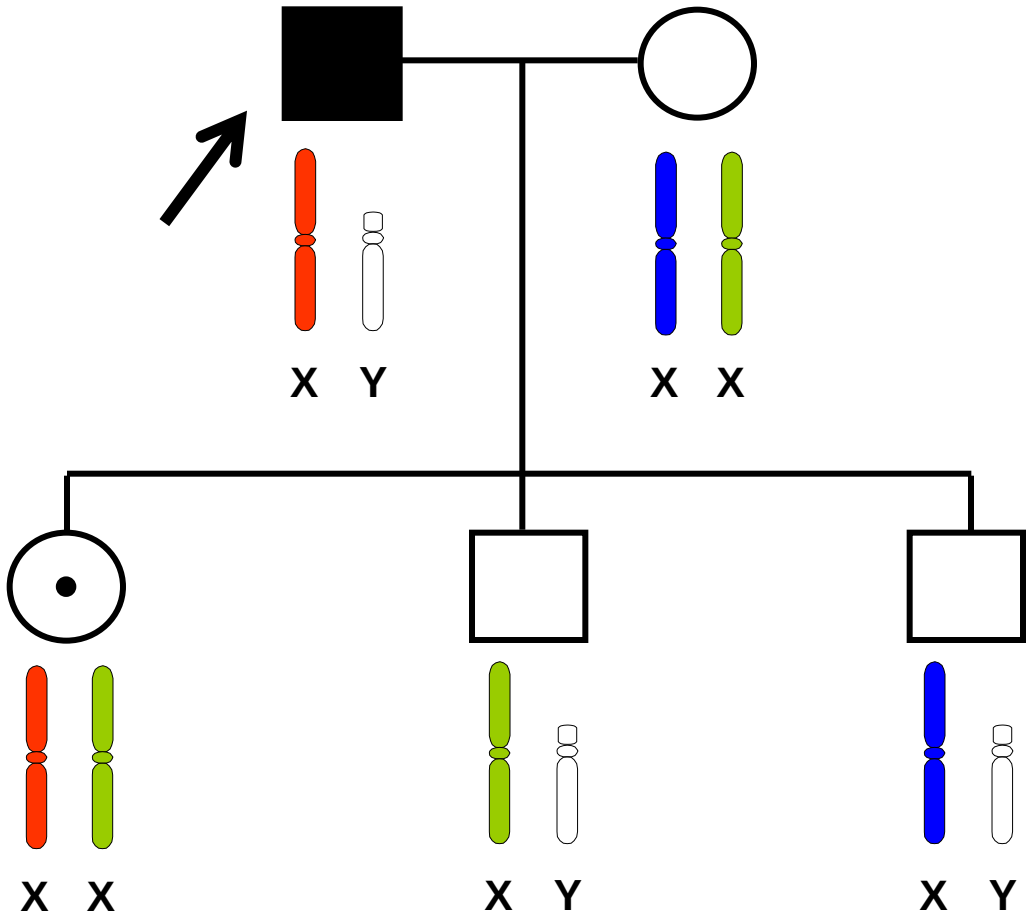
Case 2

X-linked Inheritance



Focus: Case 2

- Evaluating simplex patients
- Test selection
  - Cost
  - Yield
- X-linked inheritance

Case Vignette 3

- 35 yo woman with CMT
- Pes Cavus
- Slow NCV (20 m/s)
- Positive family history in three generations
- She requests genetic testing to confirm diagnosis and evaluate risk/diagnosis in children and other family members

Case Vignette 3

- Her Neurologist orders the full CMT panel
- Cost: $15,000
- Insurance does not cover full cost
- Patient billed for $3,000

Could this have been avoided?

1.Yes

2.No

Case Vignette 3

Could this have been avoided?

1. Yes
2. No

Case Vignette 3

TESTING ERROR

- PMP22 dup is most common cause of this syndrome (70-80%)
- PMP22 dup test is $900
- Just order the most likely test first.
- Patient indeed had PMP22 dup
- Lab should do reflexive testing
  - But many do not!

Focus: Case 3

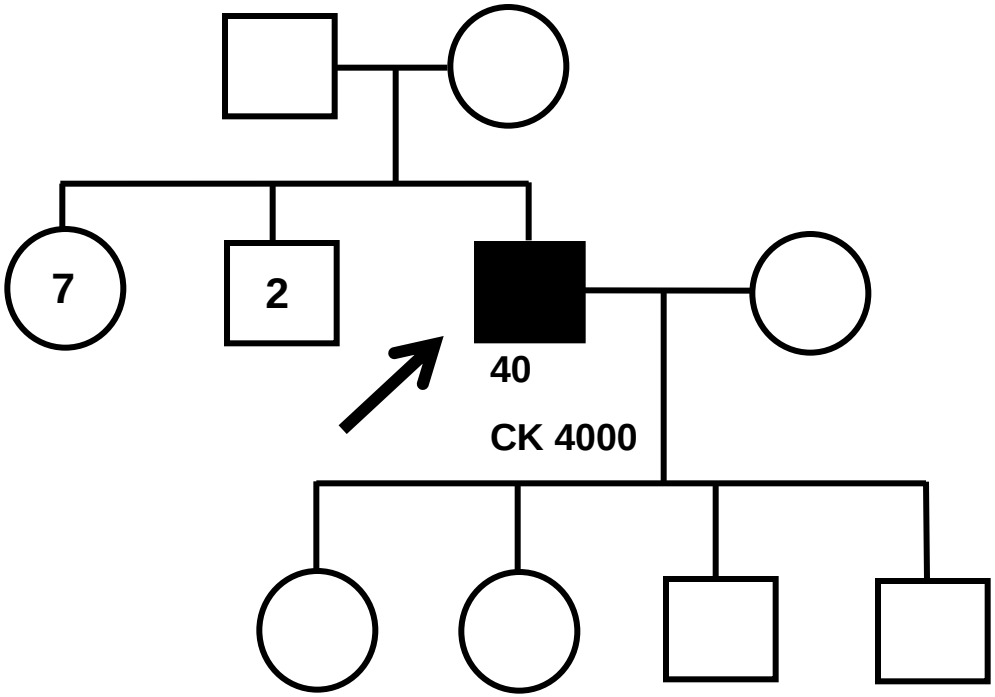
- Test for the most likely genetic cause first
- Be aware of test cost and financial implications for the patient

Case Vignette 4

40 year old man

- Muscle weakness since age 30
- Slowly progressive
- Very large calves
- CK 4,000 u
- Negative family history
- 7 sisters, 2 brothers
- 2 daughters, 2 sons

Case 4: Family History



Case Vignette 4

In 1998 at age 28 years

- *DMD* (dystrophin) gene analysis on blood
- “Multiplex PCR gene amplification” (deletion screening)
- Result: No deletion in the dystrophin gene

Case 4: Questions

- In 2010 at age 40 years

- Is there additional testing for mutations in *DMD*?

1. Yes
2. No

Case 4: Questions

- Is there additional testing for mutations in *DMD*?

1. Yes
2. No

Dystrophinopathy

Molecular Genetic Testing

Test Method	*DMD* Mutations Detected	Mutation Detection Frequency in Males by Phenotype and Test Method		
		DMD	BMD	XLDCM
Deletion / duplication testing	Deletion of one or more exons	~65%	~85%	Unknown
	Duplication of one or more exons	~6-10%	~6-10%	Unknown
Mutation scanning or sequence analysis	Small insertions/ deletions/point mutations/splicing mutations	~25-30%	~5-10%	Unknown

Case 4: Test Results

***DMD* sequence analysis**

"A to T change at nt 137 in exon 3 resulting in Asp46Val in actin binding domain."

Mutation nomenclature

c.137A>T or p.Asp46Val

Case 4: Test Results

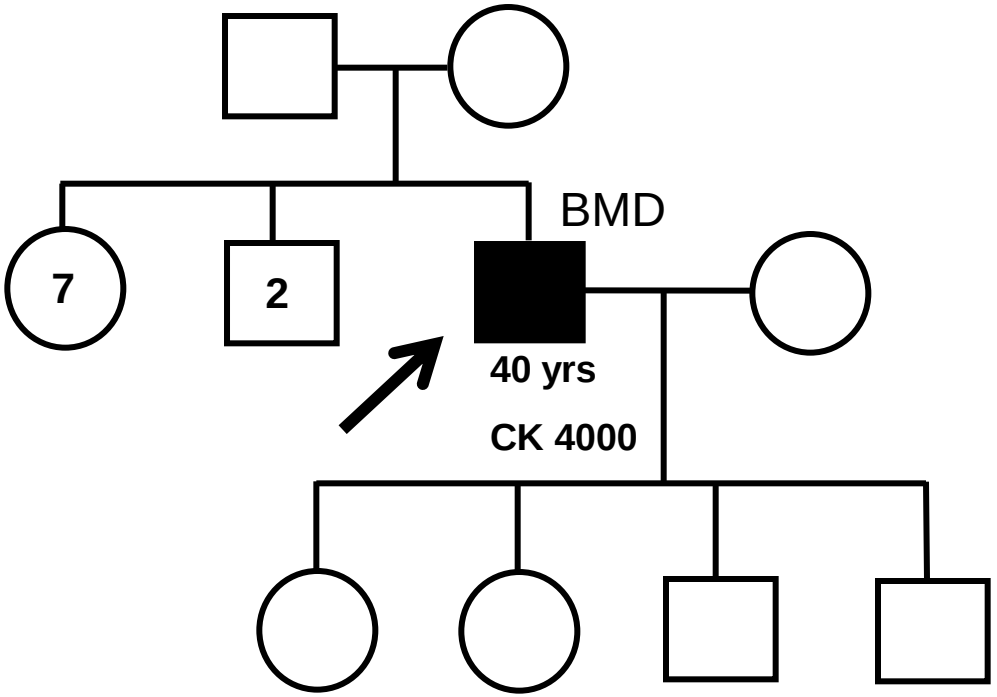
DMD testing confirms the diagnosis of Becker muscular dystrophy

Management: Needs routine cardiac evaluation

Genetic counseling:

- X-linked inheritance
- All daughters need genetic counseling & cardiac evaluations
- His mother and seven sisters could be carriers

Case 4: Family History



Case 4: Targeted mutation analysis

- Testing at-risk family members for the mutation in the proband (i.e., family-specific mutation)
  - "Family mutation evaluation"
- Must know the mutation in the proband
- Cheaper ($440) than initial test ($3800)
- Strategy: Testing mother first vs testing sisters
- Consider germline mosaicism (15%-20%)

Focus: Case 4

Test methods

- VERY likely to change over time

  Especially:

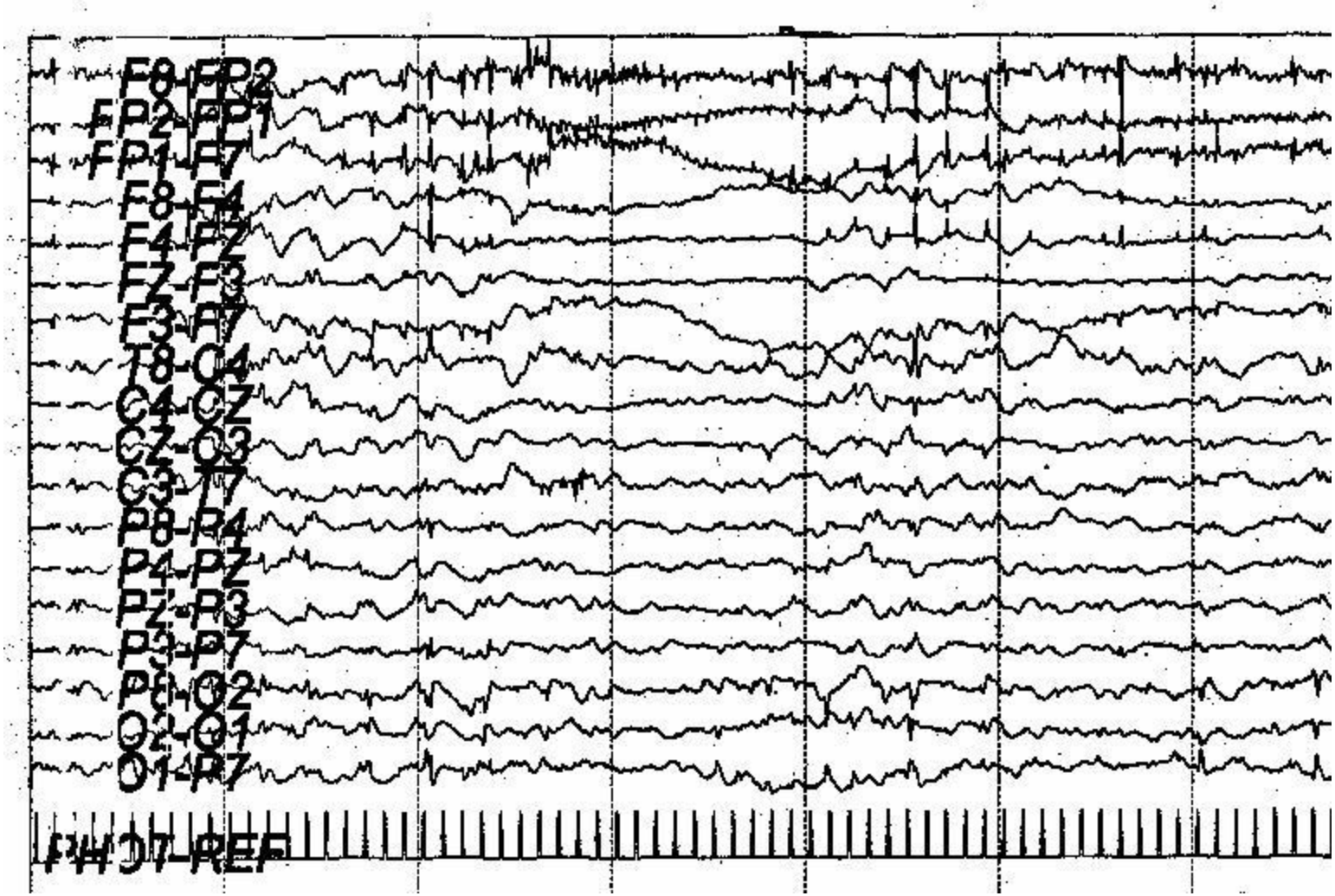
  - CMTs
  - Muscular dystrophies
  - Hereditary ataxias

- Check to see what methods are currently available

- Targeted mutation analysis

Case Vignette 5

- 12 year old girl with:
  - Recent onset stimulus sensitive myoclonus
  - Tonic-clonic seizures
  - EEG photosensitivity
  - Normal brain MRI
- Brother died at age 19 years after progressively worsening similar syndrome treated with phenytoin
- Normal parents


F8-FP2
F8-F4
FP2-FP1
FP1-F7
F8-F4
F4-F2
F2-F3
F3-F7
T8-C4
C4-CZ
CZ-C3
C3-T7
P8-P4
P4-PZ
PZ-P3
P3-P7
P8-O2
O2-O1
O1-P7
F7-REF

Case 5: Question

For which of the following reasons should genetic testing be done?

1. Diagnosis
2. Prognosis
3. Management
4. Genetic counseling
5. All of the Above

Case 5: Question

For which of the following reasons should genetic testing be done?

1. Diagnosis
2. Prognosis
3. Management
4. Genetic counseling
5. All of the Above

Case 5: Question

For which of the following disorders would you test first?

1. Tuberous sclerosis complex
2. Prion disease
3. Unverricht-Lundborg (EPM1)
4. MERRF
5. Lafora body (EPM2)

Case 5: Question

For which of the following disorders would you test first?

1. Tuberous sclerosis complex
2. Prion disease
3. Unverricht-Lundborg (EPM1)
4. MERRF
5. Lafora body (EPM2)

Case 5: Test Results

The *EPM1* gene is homozygous for a 40 dodecamer (CCC-CGC-CCC-GCG) repeat expansion

Case 5: Relevance of the Genetic Test Result

- **Diagnosis:** EPM1 (Unverricht-Lundborg Disease)
- **Prognosis:** Progressive
- **Management:** Valproate best; Phenytoin detrimental
- **Genetic counseling:** Autosomal recessive

Focus: Case 5

- Genetic testing can be clinically relevant and useful **!**

Case Vignette 6

35 year old man

- Onset ataxia age 22 yrs
- Slowly progressive
- Depressed DTR's
- Dysarthria
- ↓ Vibration in feet
- ↓ Plantar reflexes
- Normal MRI
- Negative family history

Case 6: Questions

Would you test him for Friedreich ataxia?

1. Yes
2. No

Case 6: Questions

Would you test him for Friedreich ataxia?

1. Yes

2. No

Case 6: Test Result

Friedreich ataxia molecular genetic test

- One normal allele
- One allele with **120** GAA repeats

What is the best clinical interpretation of this result?

1. Normal
2. Abnormal
3. Indeterminate

Case 6: Test Result

What is the best clinical interpretation of this result?

1. Normal
2. Abnormal
3. Indeterminate

Friedreich Ataxia

Diagnosis

Molecular Genetic Testing

Allele sizes. Four classes of alleles are recognized for the GAA triplet repeat sequence in intron 1 of the *FXN* gene.

- **Normal alleles:** 5 to 33 GAA repeats.
- **Mutable normal (premutation) alleles:** 34 to 65 pure (uninterrupted) GAA repeats.
- **Full penetrance (disease-causing expanded) alleles:** **66 to 1700** GAA repeats.
- **Borderline alleles:** 44 to 66 uninterrupted GAA repeats.

Case 6: Question

FA is an autosomal recessive disorder

– Diagnosis requires presence of two abnormal alleles

Can any other test be done to confirm or exclude FA?

1. Yes
2. No

Case 6: Question

Can any other test be done to confirm or exclude FA?

1. Yes
2. No

Friedreich Ataxia

Molecular Genetic Testing

Test Method	*FXN* Mutations Detected	Prevalence	Test Availability
Targeted mutation analysis	Homozygous GAA expansion	96%	Clinical Testing
	Heterozygous GAA expansion	4 %	
Sequence analysis	Heterozygous point mutation		

Case 6: Test Result

Sequence analysis of *FRDA* gene:

p.G130V missense mutation

Interpretation: Patient is a compound heterozygote for two abnormal alleles:

- An expanded GAA repeat
- A missense mutation

Diagnosis: Friedreich ataxia

Focus: Case 6

Test results. Presence of one mutant allele does not confirm the diagnosis of an autosomal recessive disorder.

Test methods. Additional test methods might be available to clarify an ambiguous test result.

Case Vignette 7

67 yo man with 5-10 years of numbness/weakness in both feet

- Negative neuropathy evaluation
- EMG: diffuse axonal neuropathy
- Depressed DTR's, ↓vib/position, mild bilat foot drop
- Both parents alcoholic with "balance problems"
- Daughter has 2 children with "CMT"

Case 7: *MFN2* (Mitofusin 2) Gene Sequencing

- Most common cause axonal CMT2
- DNA Transition G>A
- Nt Position: 1452
- Codon: 484
- AA change: none
- Variant Type: Variant unknown significance (VUS)

Sequence analysis

Types of sequence alterations that may be detected

- Pathogenic sequence alteration reported in the literature
- Sequence alteration predicted to be pathogenic but not reported in the literature
- Unknown sequence alteration of unpredictable clinical significance
- Sequence alteration predicted to be benign (polymorphism) but not reported in the literature
- Benign sequence alteration (polymorphism) reported in the literature

Case 7: Questions

What is the best interpretation of this result?

1. Probably a benign polymorphism
2. Definitely a causative mutation
3. Could be a causative mutation, but cannot be certain

Case 7: Questions

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1. Probably a benign polymorphism
2. Definitely a causative mutation
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Case 7: Interpretation of a sequence variant

- In coding region or splice site of gene?
- Amino acid change?
- Conserved over evolution?
- Previously reported pathogenic?
- Segregates with disease in family?

Case 7: Interpretation

- No amino acid change
- Not previously reported as pathological
- Atypical clinical story
- (Grandchildren inherited CMT from their father)
- Conclusion: A mutation in *MFN2* is not cause of neuropathy

Focus: Case 7

- DNA variants may not be pathogenic (i.e. causative)

Case Vignette 8

A 40 year old woman has a long history of peripheral neuropathy.

- Three other family members in two generations are also affected.
- Her DNA test for CMT shows a G>C at nt 487 producing a substitution of glycine for arginine at codon 163 of *MPZ,* the gene encoding myelin P zero.
- Mutation is c.487G>C (p.R163G)

MPZ Gene

Mutation = G163R

Codon 163			Amino Acid
G*	G	U	Gly (G)
↓			
C	G	U	Arg (R)

*Nucleotide 487

Case 8: Test Results

The laboratory says this is an “indeterminate result” because it has not been previously reported.

Variant of unknown significance (VOUS)

Case 8: Questions

What is the best interpretation of this result?

1. Probably a benign polymorphism
2. Definitely a causative mutation
3. Could be a causative mutation, but cannot be certain

Case 8: Questions

What is the best interpretation of this result?

1. Probably a benign polymorphism
2. Definitely a causative mutation
3. Could be a causative mutation, but cannot be certain

Case 8: Questions

Will any further testing help?

1. Yes
2. No

Case 8: Questions

Will any further testing help?

1. Yes

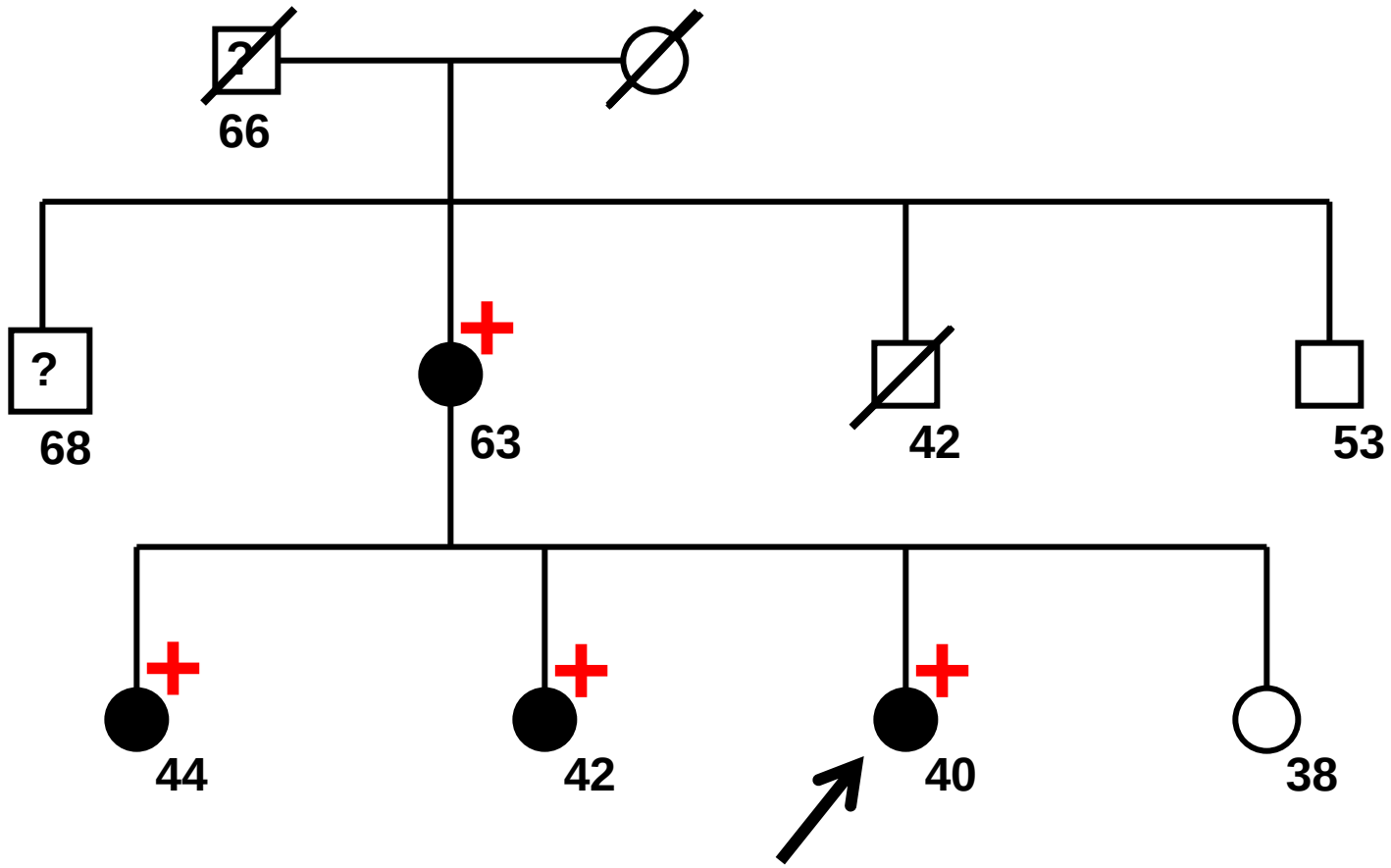
2. No

Case 8:
Interpretation of test results

- Targeted mutation analysis
- Determine how the mutation segregates with the phenotype in the family
- Mutation = Sequence variant; does not imply pathology

Case 8: Interpretation of test results

Determine how the mutation segregates with the phenotype in the family



\+ G163R

● CMT

Muscle Nerve. 2004 Jun;29(6):867-9.

Clinical and genetic description of a family with Charcot-Marie-Tooth disease type 1B from a transmembrane MPZ mutation.

Eggers SD, Keswani SC, Melli G, Cornblath DR.

Abstract

Mutations in the myelin protein zero gene (MPZ) are associated with certain demyelinating neuropathies, and in particular with Charcot-Marie-Tooth disease type 1B (CMT1B), Dejerine-Sottas syndrome, and congenital hypomyelination. MPZ mutations affecting the protein's transmembrane domain are generally associated with more severe phenotypes. We describe a family with mild CMT1B associated with a transmembrane MPZ mutation. Sequence analysis identified a G-to-C transversion at nucleotide 1064, predicting a glycine-to-arginine **substitution in codon 163 (G163R) of MPZ**. This report furthers the understanding of the clinical and electrophysiological manifestations of MPZ mutations.

PMID: 15170620

Interpreting DNA variants

Benign	Unknown					Pathogenic
				X		

Variant: SETX c.1398 T>G (p,Ile466Met)
Segregation Analysis: No information available
Co-occurrence: Not enough information
General pop. freq.: No information available
Amino Acid Conservation: Moderately conserved across species
Grantham Score: 10 [0-215] (conservative difference)
SIFT: Predicted NOT Tolerated
PolyPhen-2 (HumVar): Probably Damaging
Protein Domain: N-terminus
dbSNP Reference: None

Focus: Case 8

Test results

The laboratory may be able to offer additional testing to clarify indeterminate results, such as variants of unknown significance (VOUS)

Case Vignette 9

42 year old woman with family history of Huntington disease.

- She has a normal exam and requests presymptomatic testing for HD
- Father, uncle and younger sister had progressive neurologic disease; all are deceased.
- Sister's brain at autopsy reported as "compatible with HD." Frozen brain tissue was saved.

Case 9: Question

Would you test this woman for the repeat expansion in the *HTT* gene associated with HD?

1. Yes
2. No

Case 9: Question

Would you test this woman for the repeat expansion in the *HTT* gene associated with HD?

1. Yes
2. No

Case 9

No! You should not test an asymptomatic at-risk relative without a molecular diagnosis in an affected family member.

Htt testing on frozen tissue from the deceased sister was normal.

Case 9

More family history was obtained:

- The deceased sister also had visual loss resulting in blindness
- Records on other family members showed a diagnosis of severe cerebellar ataxia

Case Vignette 9

For which of the following genetic disorders would you test the frozen tissue?

1. SCA 1
2. SCA 2
3. SCA 3
4. SCA 6
5. SCA 7

Case Vignette 9

For which of the following genetic disorders would you test the frozen tissue?

1. SCA 1
2. SCA 2
3. SCA 3
4. SCA 6
5. SCA 7

Case 9: Test Results

The tissue had 80 CAG repeats in the *SCA7* gene

Spinocerebellar Ataxia Type 7

Molecular genetic testing

Allele sizes

- **Normal alleles:** 19 or fewer CAG repeats.
- **Mutable normal alleles:** 30 to 35 repeats
- **Reduced penetrance alleles:** alleles with 34-36 repeats may be provisionally defined as alleles with reduced penetrance
- **Full penetrance alleles:** **36** to **460** CAG repeats.

Case 9: Test Results

The consultand tests normal for the *SCA 7* CAG repeat

Case 9: Focus

Testing strategy in a family.

You must confirm the diagnosis in an affected relative before offering presymptomatic testing to at-risk family members.

Case Vignette 10

45 year old man with:

- Intellectual Impairment
- Retinitis pigmentosa
- Cerebellar ataxia

  - Cerebellar atrophy on MRI

- Peripheral neuropathy

  - Pes cavus with foot drop

- Negative family history (simplex case)

Case 10: Question

What genetic category best fits this case?

1. Autosomal Dominant
2. Autosomal Recessive
3. Mitochondrial
4. X-linked
5. Polygenic

Case 10: Question

What genetic category best fits this case?

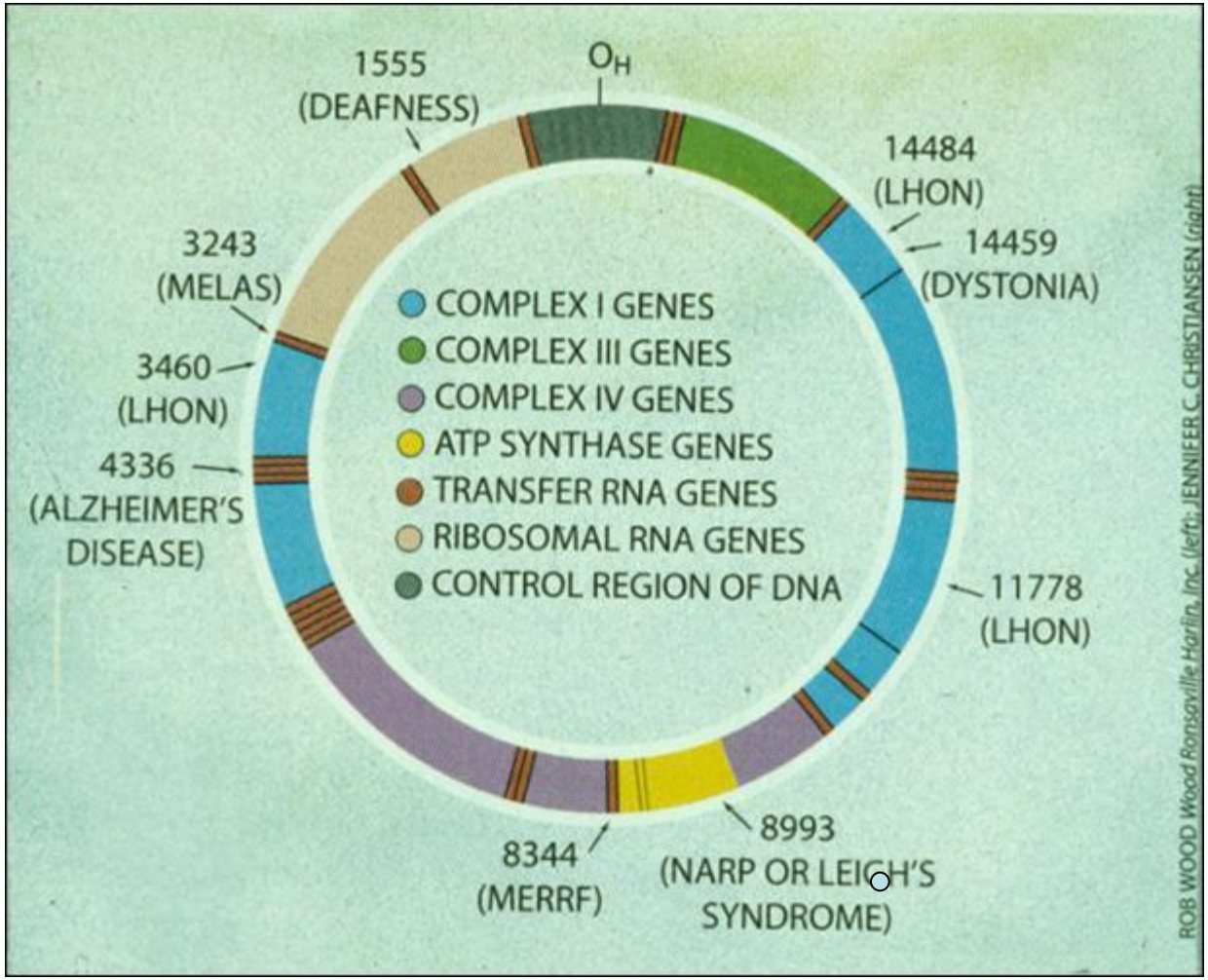
1. Autosomal Dominant
2. Autosomal Recessive
3. Mitochondrial
4. X-linked
5. Polygenic

Case 10

- **N**europathy
- **A**taxia
- **R**etinitis **P**igmentosa

NARP

Mitochondrial Genome



NARP-Leigh syndrome continuum: mtDNA Targeted Mutation Analysis*

- T > G conversion single
- nt 8993
- T 8993 G

*White cell DNA

NARP-Leigh syndrome continuum: mtDNA Targeted Mutation Analysis*

Gene: *MT-ATP6*

- T>G single nt substitution at position 8993 of entire mitochondrial genome (~16 kb)
- Nucleotide change: m.8993T>G
  - Prefix “m”=mitochondrial reference sequence
- Protein amino acid change: p.Leu156Arg

*White cell DNA

NARP-Leigh syndrome continuum

Leigh Syndrome

- Infantile onset
- Subacute relapsing encephalopathy
- Cerebellar and brain-stem signs

NARP

- Late-childhood or adult-onset
- Sensorimotor neuropathy
- Ataxia
- Pigmentary retinopathy

Both

- Basal ganglia lucencies

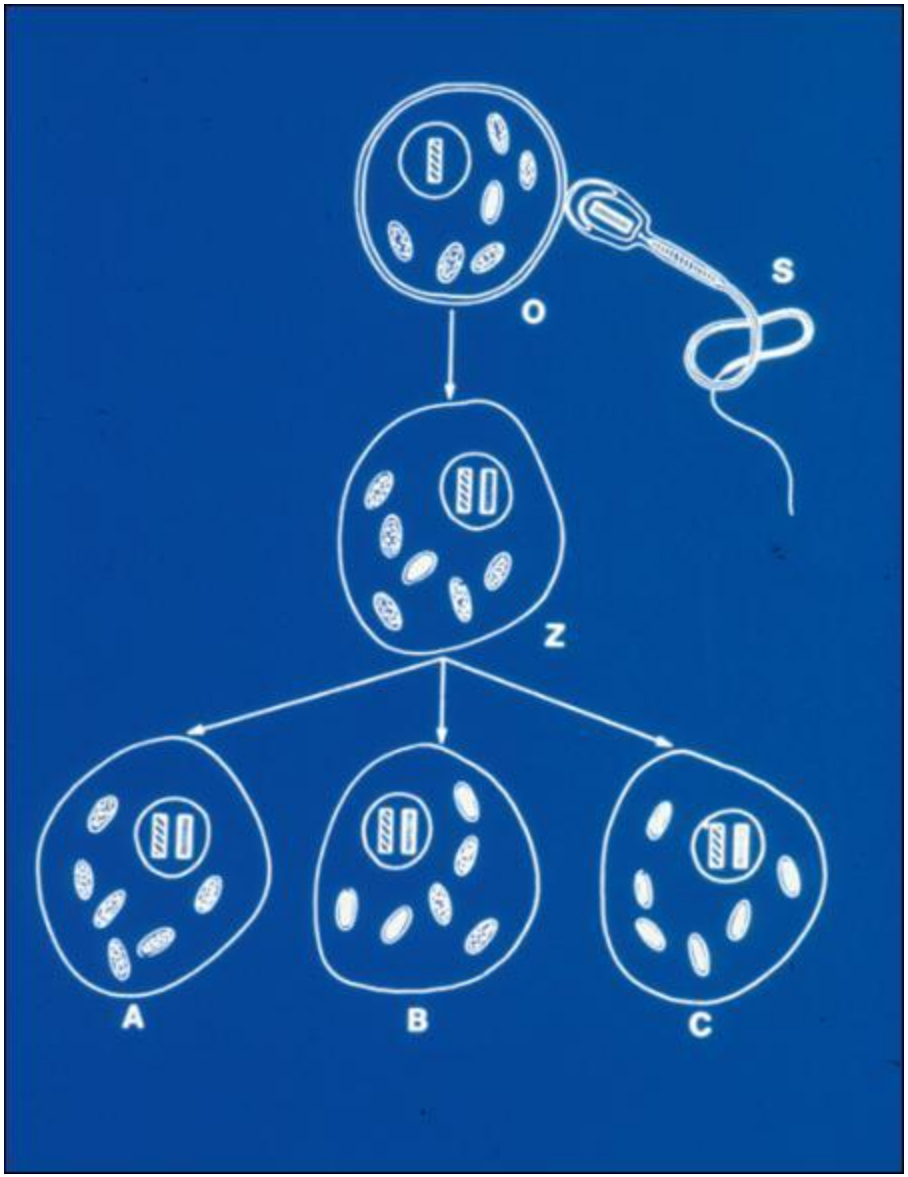
Case 10: Mitochondrial Inheritance

Genetic risks to this man's:

- Children
- Sister
- Sister's children
- Mother

Mitochondrial DNA

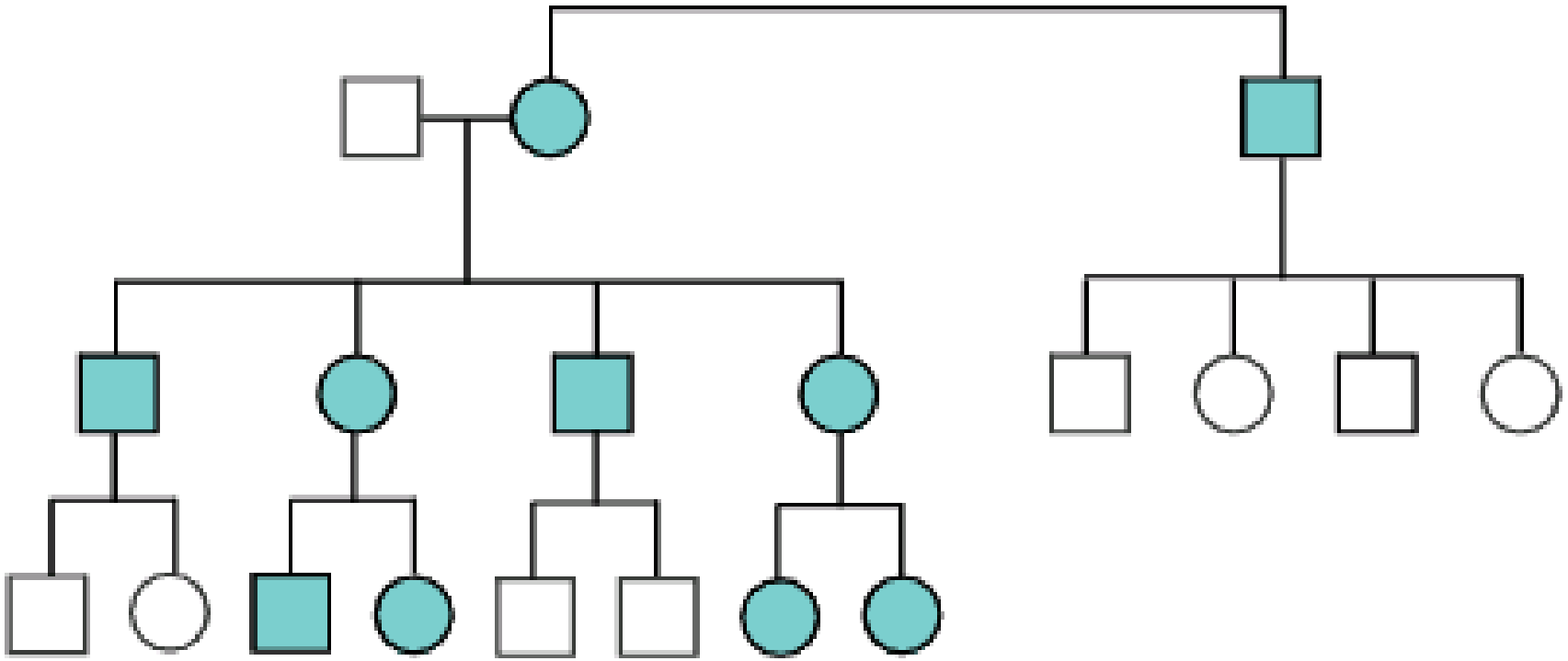
Sperm: None
Egg: 100%



Inheritance of a Mitochondrial Disorder

Note: Affected females transmit the disease to all their children.

Note: Affected males do not transmit the disease to their children.

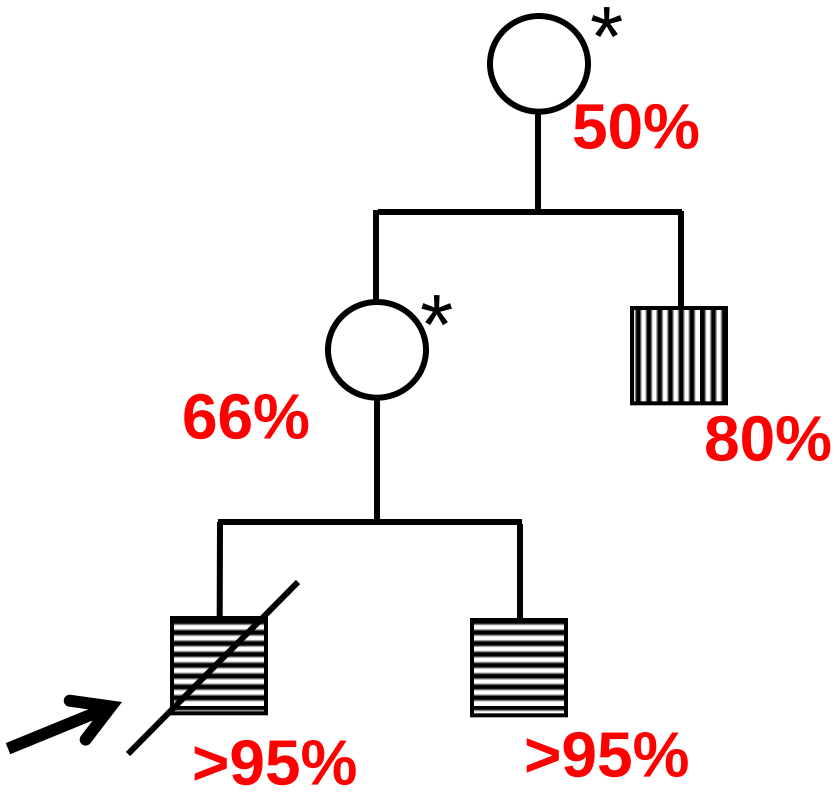


Mitochondrial Inheritance: Heteroplasmy

Heteroplasmy: Ratio of mutant mtDNA to normal mtDNA varies from cell to cell

- Does a close relationship exist between the mtDNA mutant load and disease severity?
- Is mutant mtDNA uniformly distributed in all tissues?
- Does mutant load change with time?

NARP-Leigh syndrome continuum



Leigh disease

NARP

* Clinically normal

% mtDNA with 8993 mutation

Case 10: Mitochondrial Inheritance

Genetic risks to this man's children?

1. None
2. 50%
3. 25%
4. 10%

Case 10: Mitochondrial Inheritance

Genetic risks to this man's children?

1. None
2. 50%
3. 25%
4. 10%

Case 10: Mitochondrial Inheritance

Genetic risks to this man's mother, sister and sister's children?

1. None
2. Difficult to determine
3. 50%
4. 25%

Case 10: Mitochondrial Inheritance

Genetic risks to this man's mother, sister and sister's children?

1. None
2. Difficult to determine
3. 50%
4. 25%

Focus: Case 10

Mitochondrial Inheritance

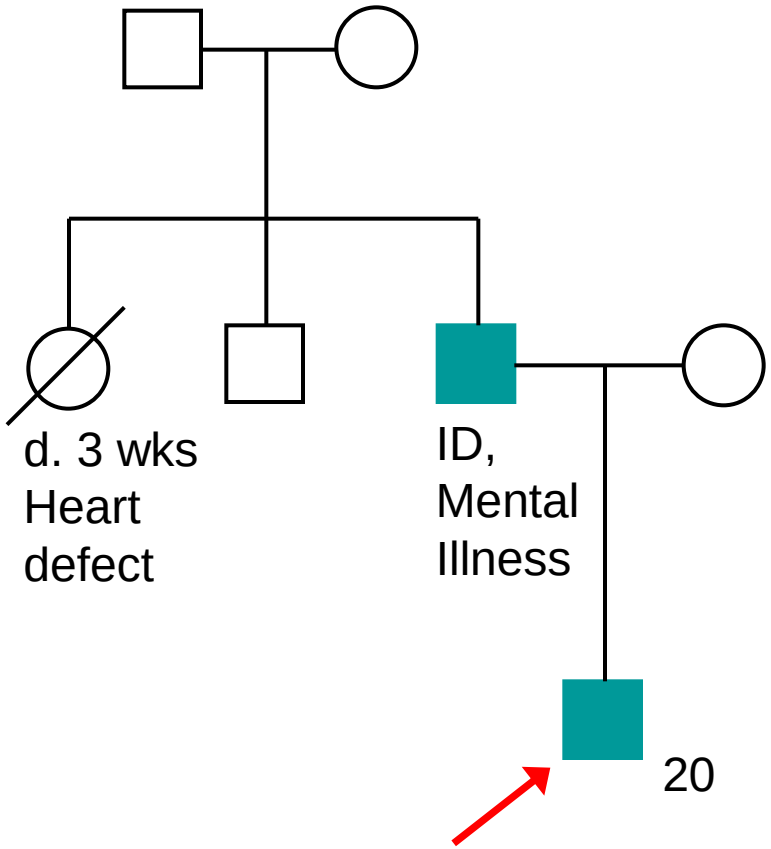
Genetic counseling implications of difficulty in predicting phenotype

- Relatives at risk
- Prenatal testing

Case 11: 20 yo Male

- Learning disabilities
- Schizoid personality disorder
- Short stature (<3rd centile)
- Prenatal etoh and drug exposure
- Premature birth
- Bilateral ureter abnormality
- Retrognathia with “distinctive” facial features
- Father has ID and mental illness

Case 11



Case 11

Appropriate testing for the patient would be:

1. Karyotype
2. Array CGH
3. Fragile X DNA test
4. Unlikely to be genetic

Case 11

Appropriate testing for the patient would be:

1. Karyotype
2. Array CGH
3. Fragile X DNA test
4. Unlikely to be genetic

Case 11

Chromosomal microarray determination of copy number variants (CNV) as approach to complex syndromes and congenital abnormalities.

- Developmental Delay
- Cognitive Impairment
- Autism
- Seizures

Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies

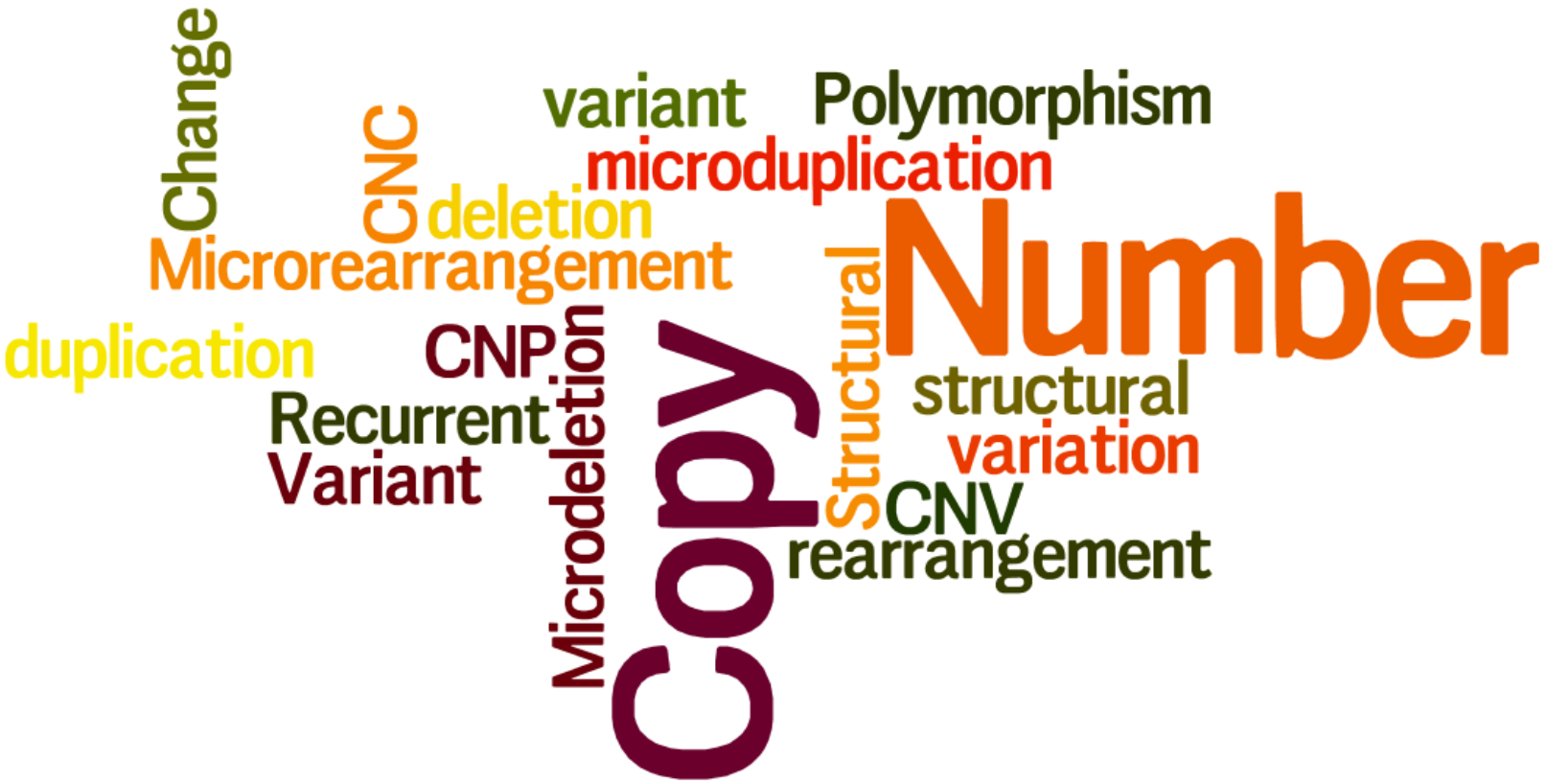
Miller DT, Adam MP, Aradhya S, et al

Am J Hum Genet 2010; 86: 749-764

Copy number variation

- Difference in # of copies of a genomic segment
  - Deletion
  - Duplication
  - Insertion
- Size can vary
  - Usually defined as >1 kb
  - Can be up to several Mb

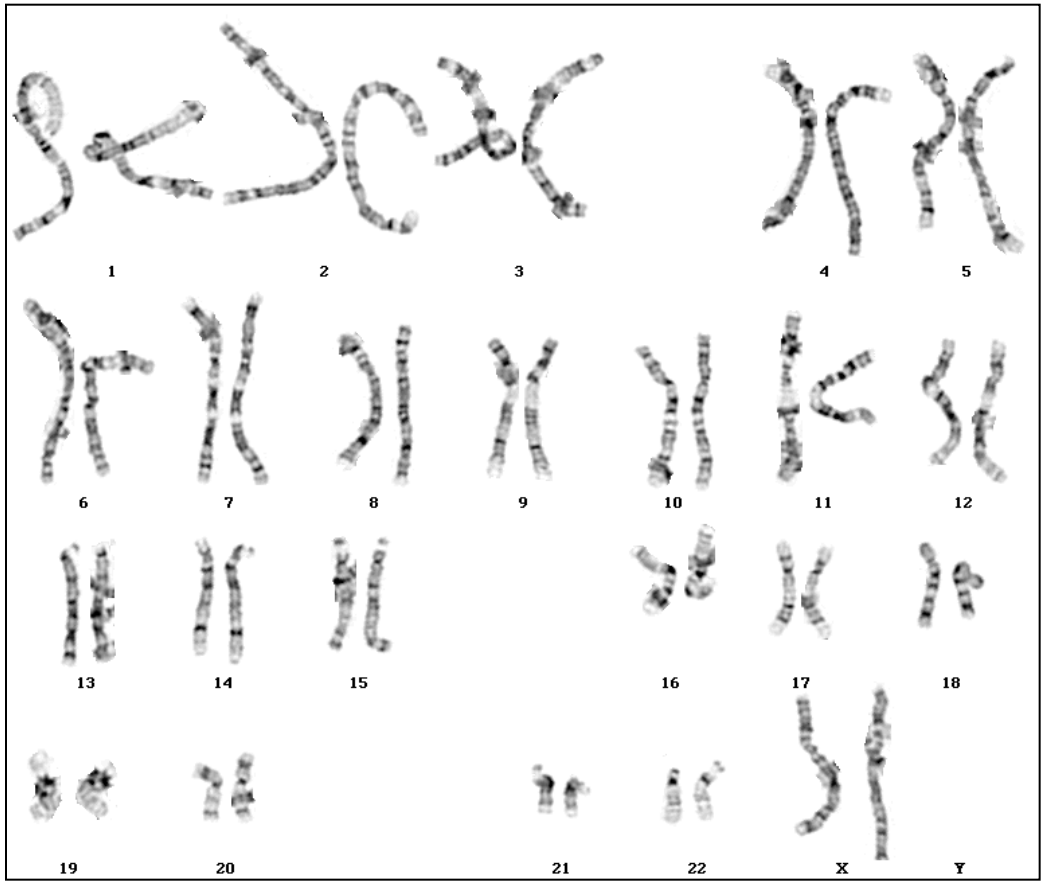
Jargon


Change
CNC
variant
Polymorphism
microduplication
deletion
Number
Microrearrangement
Structural
duplication
CNP
Microdeletion
Copy
structural
Recurrent
variation
Variant
CNV
rearrangement

Copy number variation

- CNV = Copy Number Variant
- CNP = Copy Number Polymorphism (>1%)
- CNC = Copy Number Change
- Microdeletion / microduplication
- Structural variation / variant
- Microrearrangement
- Recurrent rearrangement
- Chromosomal imbalance

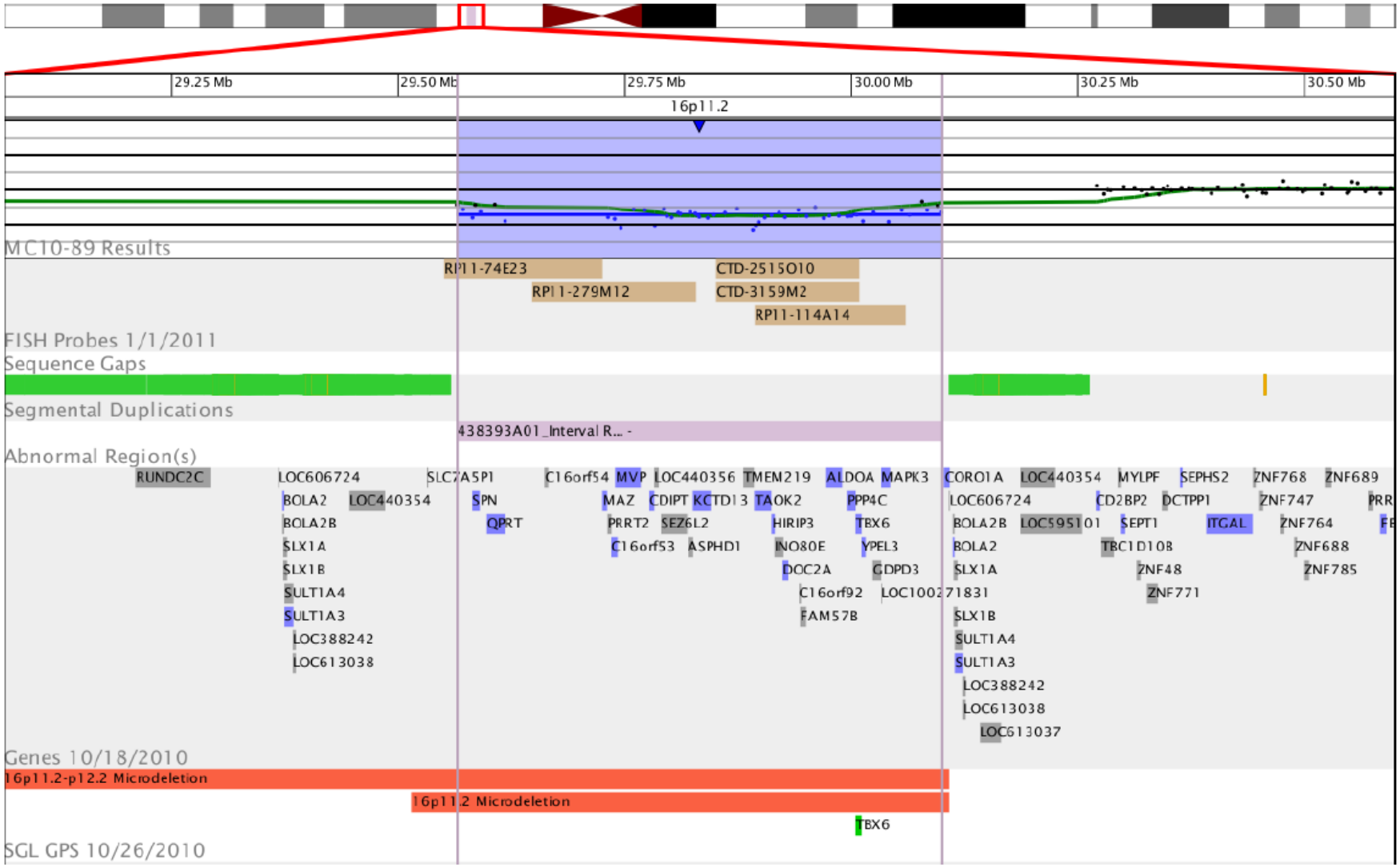
Chromosome analysis



Conventional karyotype analysis has a resolution of ~5-10 Mb

Most of these changes are pathogenic

Case 11:
Array CGH - 16p11.2 Del



Result Provides Diagnosis

Discovery of a previously unrecognized microdeletion syndrome of 16p11.2–p12.2

Blake C Ballif[1], Sara A Hornor[2], Elizabeth Jenkins[3], Suneeta Madan-Khetarpal[3], Urvashi Surti[4,5], Kelly E Jackson[6], Alexander Asamoah[6], Pamela L Brock[6], Gordon C Gowans[6], Robert L Conway[7], John M Graham, Jr[7], Livija Medne[8], Elaine H Zackai[8], Tamim H Shaikh[8], Joel Geoghegan[9], Rebecca R Selzer[9], Peggy S Eis[9], Bassem A Bejjani[1,2,10] & Lisa G Shaffer[1,2]

We have identified a recurrent *de novo* pericentromeric deletion in 16p11.2–p12.2 in four individuals with developmental disabilities by microarray-based comparative genomic hybridization analysis. The identification of common clinical features in these four individuals along with the characterization of complex segmental duplications flanking the deletion regions suggests that nonallelic homologous recombination mediated these rearrangements and that deletions in 16p11.2–p12.2 constitute a previously undescribed syndrome.

Nat Genet. 2007 Sep;39(9):1071-3

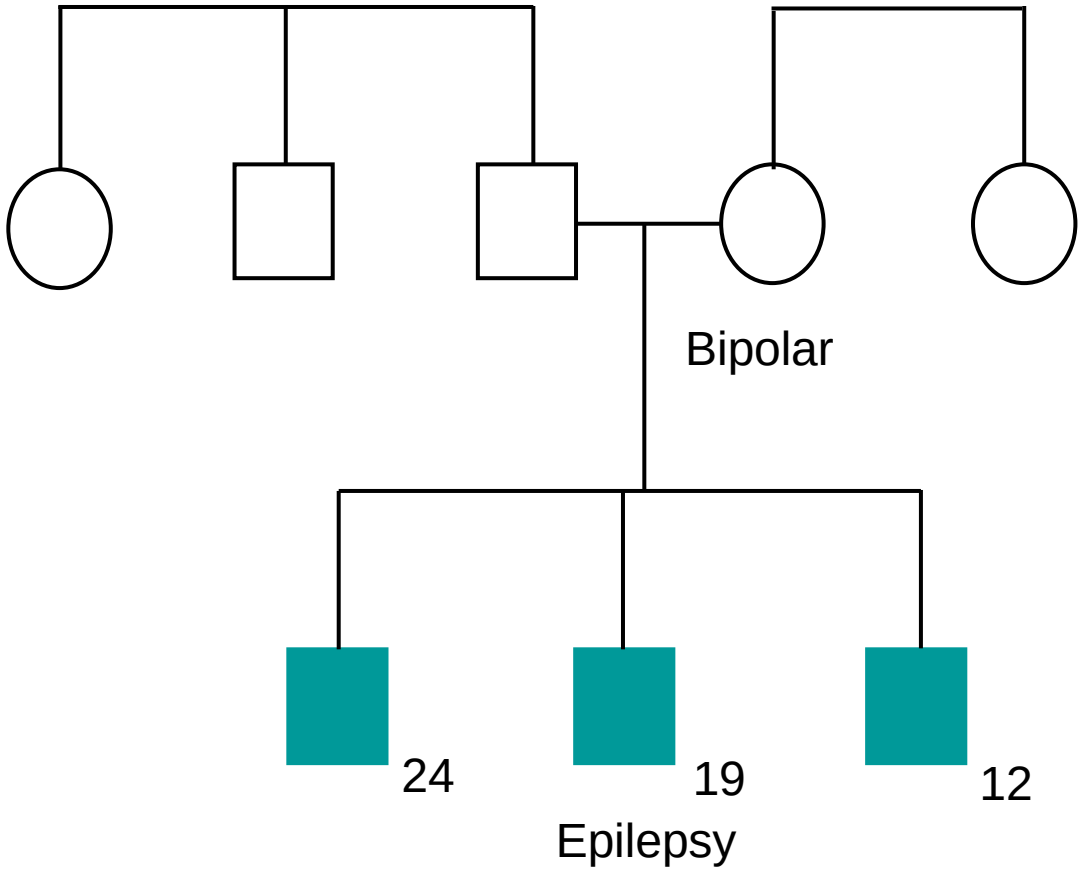
Case 12: 3 Brothers with Autism

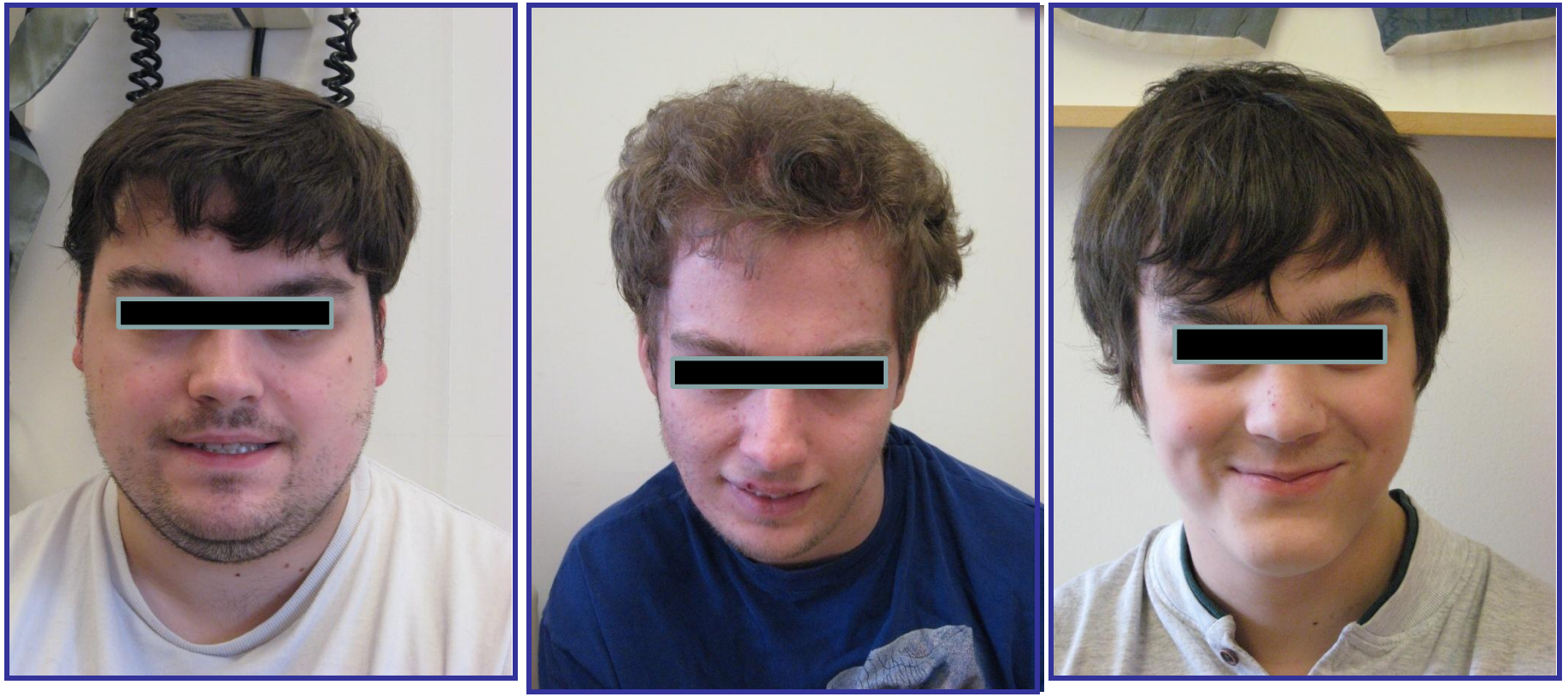
Features in all 3:

- Learning disabilities
- Meet diagnostic criteria for Autism Spectrum Disorder
- Gynecomastia
- Normal stature and appearance

Middle brother with seizure onset at 17 mo cognitive decline, averbal, nml brain MRI

Case 12





Slide courtesy of Dr. F. Hisama

Case 12:
Array CGH - Xp11.21 Dup

Only 1 Case Report

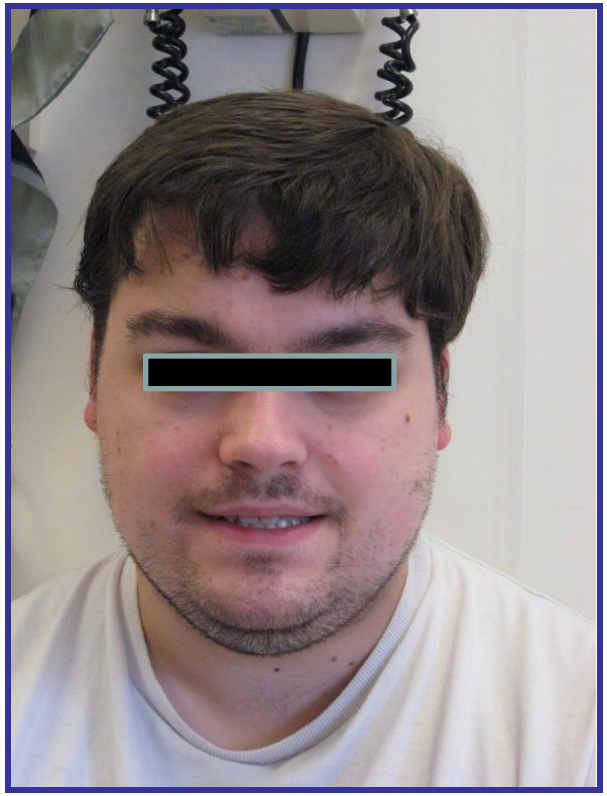
SHORT REPORT

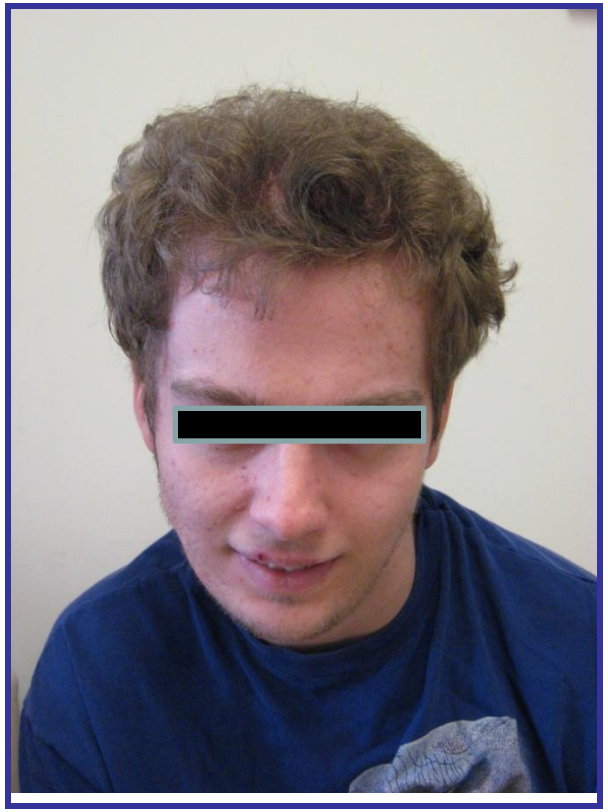
Abnormal expression of the *KLF8* (*ZNF741*) gene in a female patient with an X;autosome translocation t(X;21)(p11.2;q22.3) and non-syndromic mental retardation

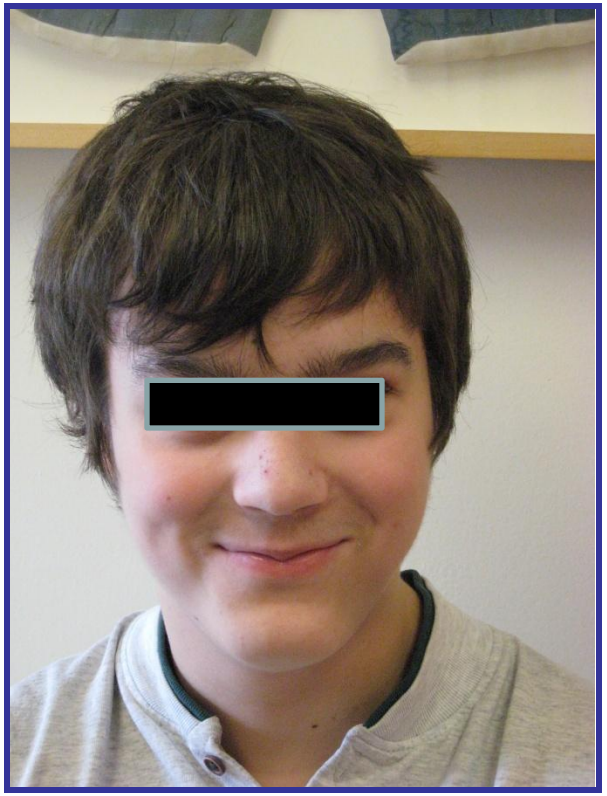
A-M Lossi, F Laugier-Anfossi, D Depetris, J Gecz, A Gedeon, F Kooy, C Schwartz, M-G Mattei, M-F Croquette, L Villard

J Med Genet 2002;**39**:113–117

And it's translocation, not duplication, in female





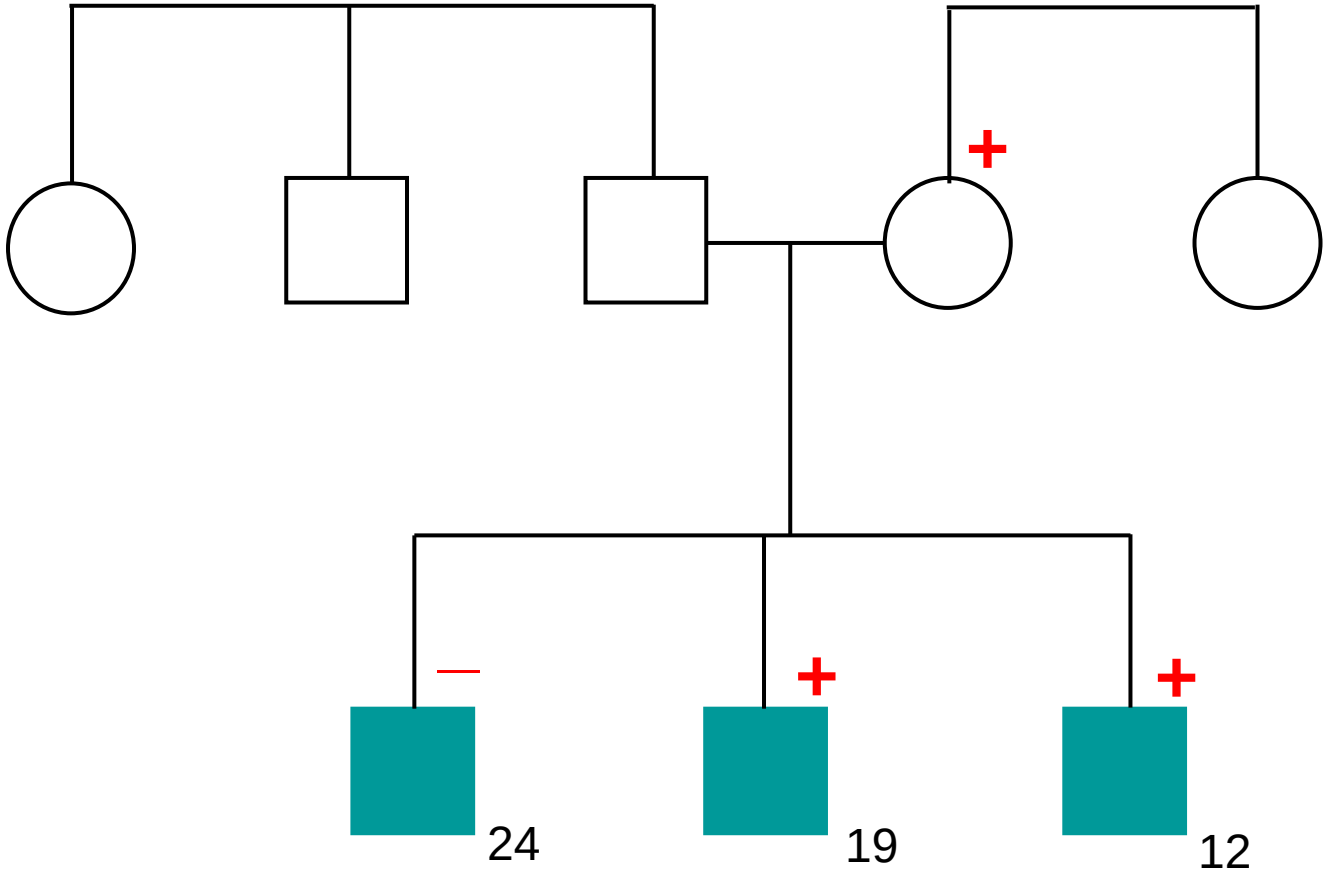


VUS — + +

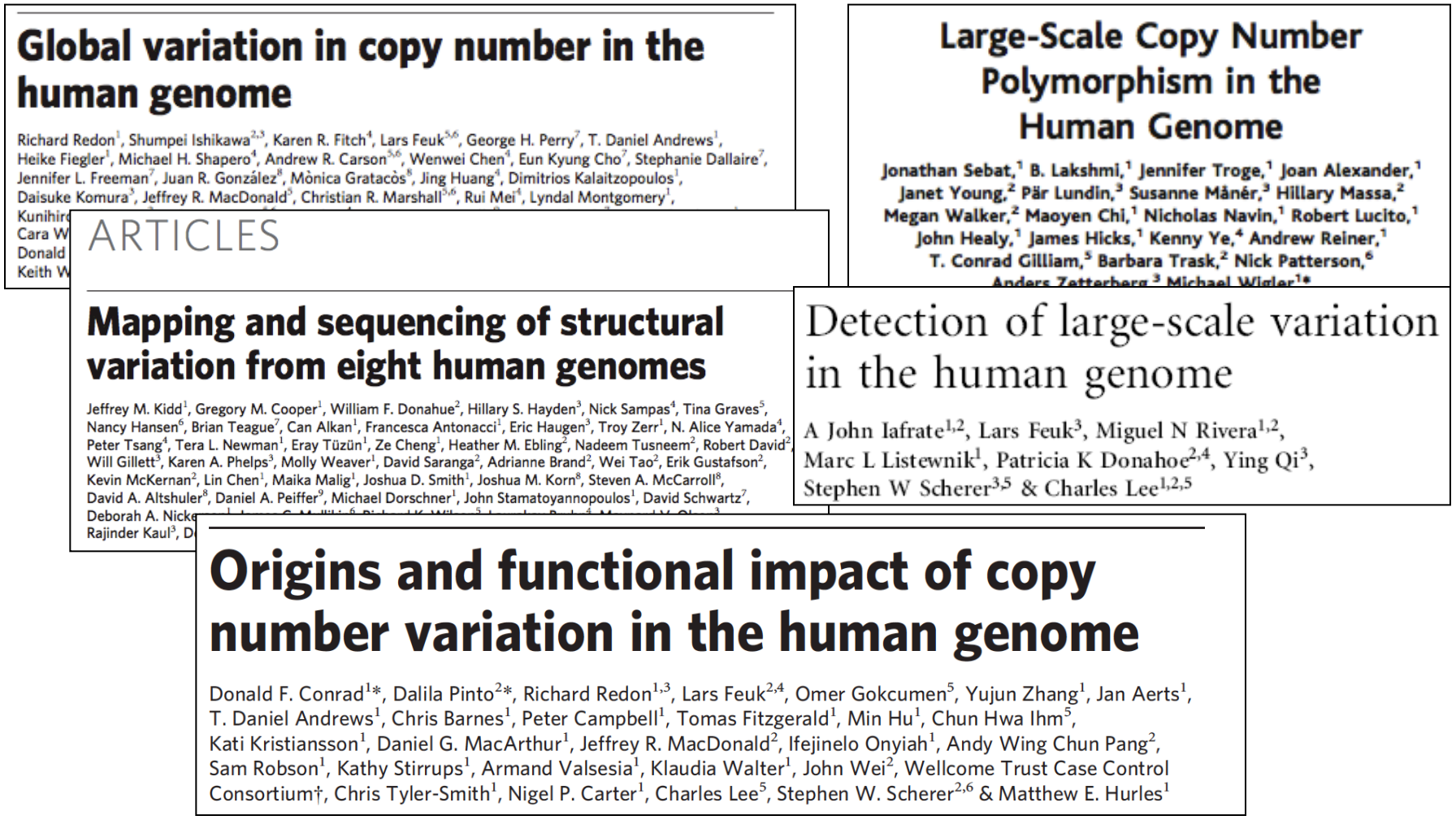
Mother + Father —

Slide courtesy of Dr. F. Hisama

Does CNV Cause Phenotype?



CNVs in healthy individuals



Global variation in copy number in the human genome

Richard Redon[1], Shumpei Ishikawa[2,3], Karen R. Fitch[4], Lars Feuk[5,6], George H. Perry[7], T. Daniel Andrews[1], Heike Fiegler[1], Michael H. Shapero[4], Andrew R. Carson[5,6], Wenwei Chen[4], Eun Kyung Cho[7], Stephanie Dallaire[7], Jennifer L. Freeman[7], Juan R. González[8], Mònica Gratacòs[8], Jing Huang[4], Dimitrios Kalaitzopoulos[1], Daisuke Komura[5], Jeffrey R. MacDonald[5], Christian R. Marshall[5,6], Rui Mei[4], Lyndal Montgomery[1], Kunihir… Cara W… Donald … Keith W…

Large-Scale Copy Number Polymorphism in the Human Genome

Jonathan Sebat,[1] B. Lakshmi,[1] Jennifer Troge,[1] Joan Alexander,[1] Janet Young,[2] Pär Lundin,[3] Susanne Månér,[3] Hillary Massa,[2] Megan Walker,[2] Maoyen Chi,[1] Nicholas Navin,[1] Robert Lucito,[1] John Healy,[1] James Hicks,[1] Kenny Ye,[4] Andrew Reiner,[1] T. Conrad Gilliam,[5] Barbara Trask,[2] Nick Patterson,[6] Anders Zetterberg,[3] Michael Wigler[1]*

ARTICLES

Mapping and sequencing of structural variation from eight human genomes

Jeffrey M. Kidd[1], Gregory M. Cooper[1], William F. Donahue[2], Hillary S. Hayden[3], Nick Sampas[4], Tina Graves[5], Nancy Hansen[6], Brian Teague[7], Can Alkan[1], Francesca Antonacci[1], Eric Haugen[3], Troy Zerr[1], N. Alice Yamada[4], Peter Tsang[4], Tera L. Newman[1], Eray Tüzün[1], Ze Cheng[1], Heather M. Ebling[2], Nadeem Tusneem[2], Robert David[2], Will Gillett[3], Karen A. Phelps[3], Molly Weaver[1], David Saranga[2], Adrianne Brand[2], Wei Tao[2], Erik Gustafson[2], Kevin McKernan[2], Lin Chen[1], Maika Malig[1], Joshua D. Smith[1], Joshua M. Korn[8], Steven A. McCarroll[8], David A. Altshuler[8], Daniel A. Peiffer[9], Michael Dorschner[1], John Stamatoyannopoulos[1], David Schwartz[7], Deborah A. Nicke… Rajinder Kaul[3], D…

Detection of large-scale variation in the human genome

A John Iafrate[1,2], Lars Feuk[3], Miguel N Rivera[1,2], Marc L Listewnik[1], Patricia K Donahoe[2,4], Ying Qi[3], Stephen W Scherer[3,5] & Charles Lee[1,2,5]

Origins and functional impact of copy number variation in the human genome

Donald F. Conrad[1]*, Dalila Pinto[2]*, Richard Redon[1,3], Lars Feuk[2,4], Omer Gokcumen[5], Yujun Zhang[1], Jan Aerts[1], T. Daniel Andrews[1], Chris Barnes[1], Peter Campbell[1], Tomas Fitzgerald[1], Min Hu[1], Chun Hwa Ihm[5], Kati Kristiansson[1], Daniel G. MacArthur[1], Jeffrey R. MacDonald[2], Ifejinelo Onyiah[1], Andy Wing Chun Pang[2], Sam Robson[1], Kathy Stirrups[1], Armand Valsesia[1], Klaudia Walter[1], John Wei[2], Wellcome Trust Case Control Consortium†, Chris Tyler-Smith[1], Nigel P. Carter[1], Charles Lee[5], Stephen W. Scherer[2,6] & Matthew E. Hurles[1]

H. Mefford, 2011

“Normal” CNVs

ARTICLE

Population Analysis of Large Copy Number Variants and Hotspots of Human Genetic Disease

Andy Itsara,[1,7] Gregory M. Cooper,[1,7] Carl Baker,[1] Santhosh Girirajan,[1] Jun Li,[2] Devin Absher,[3] Ronald M. Krauss,[4] Richard M. Myers,[3] Paul M. Ridker,[5] Daniel I. Chasman,[5] Heather Mefford,[1] Phyllis Ying,[1] Deborah A. Nickerson,[1] and Evan E. Eichler[1,6,*]

Am J Hum Genet. 2009 Feb;84(2):148-61. Epub 2009 Jan 22

- 65%–80% of individuals have CNV of at least 100 kb
- 5%–10% of individuals have CNV of at least 500 kb
- At least 1% of individuals have CNV ≥ 1 Mb

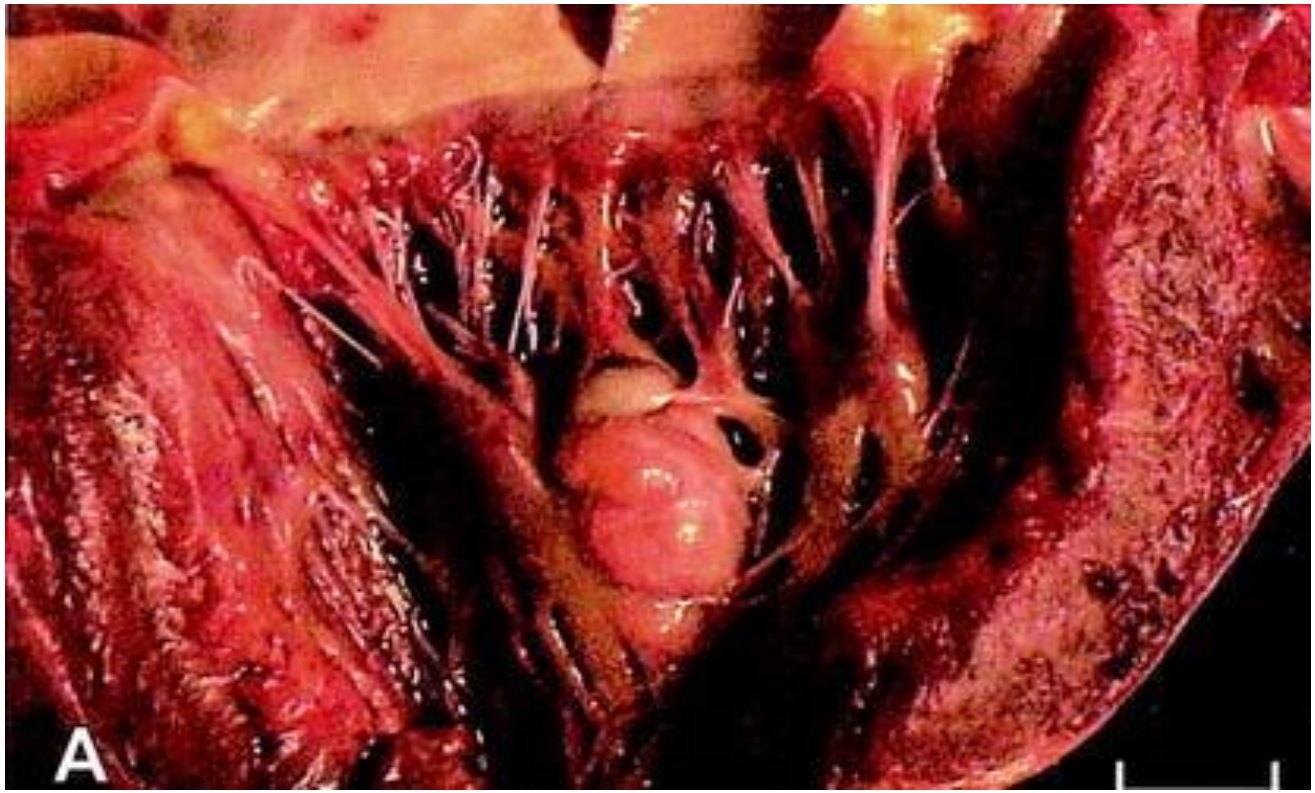
H. Mefford, 2011

Focus Cases 11 & 12

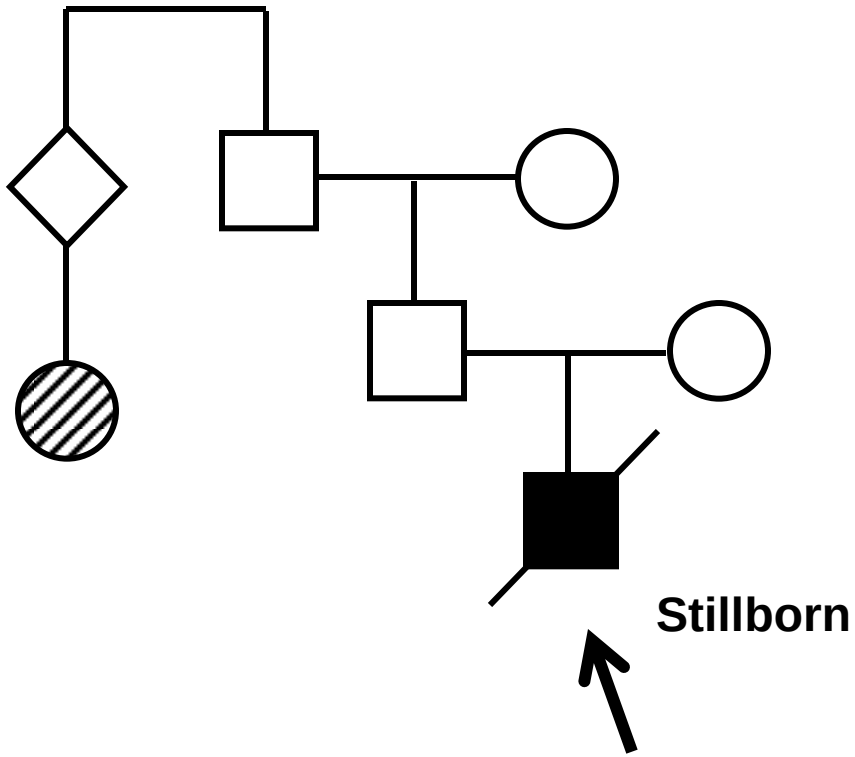
- Indications for chromosomal microarray testing
- First Tier Test
- CNV are common
- Interpretation must be individualized

Case Vignette 13

- A male diagnosed prenatally to have cardiac rhabdomyoma is stillborn
- His parents are healthy
- The father has a 30 yo paternal female first cousin with a seizure disorder and pulmonary disease



Case Vignette 13



Cardiac rhabdomyoma

Seizures; pulmonary disease

Case 13: Question

Which of the following disorders best explains the findings in this family?

1. Neurofibromatosis type 1
2. Tuberous sclerosis complex
3. Von Hippel Lindau disease
4. Myotonic dystrophy type 1

Case 13: Question

Which of the following disorders best explains the findings in this family?

1. Neurofibromatosis type 1
2. Tuberous sclerosis complex
3. Von Hippel Lindau disease
4. Myotonic dystrophy type 1

Case 13: Question

Which of the following is the next best step in the evaluation of this family?

1. Examine and test the parents of the stillborn
2. Examine the parents and test tissue from the stillborn
3. Examine and test the cousin

Case 13: Question

Which of the following is the next best step in the evaluation of this family?

1. Examine and test the parents of the stillborn
2. Examine the parents and test tissue from the stillborn
3. Examine and test the cousin

Case 13: Results

- Skin and eye examinations, brain MRIs and renal US examinations are normal in both parents

- Tissue from the stillborn shows a missense mutation in the *TSC1* gene

- What should you do next?

    1. Test the mother
    2. Test the father
    3. Test both parents

Case 13: Results

What should you do next?

1. Test the mother
2. Test the father
3. Test both parents

Case 13: Results

- The father has the same *TSC1* missense mutation as the stillborn
- The mother does not have a *TSC1* mutation
- The father's cousin has pulmonary lymphangiomyomatosis (LAM) and the same *TSC1* mutation.

Case 13: Results

How do you explain the findings in this family?

1. Variable expressivity
2. Reduced penetrance
3. Both

Case 13: Results

How do you explain the findings in this family?

1. Variable expressivity
2. Reduced penetrance
3. Both

Case 13

Variable expressivity: Variation in clinical features (type and severity) of a genetic disorder between affected individuals, even within the same family

Reduced penetrance: The proportion of individuals with a mutation causing a disorder who exhibit clinical symptoms is less than 100%

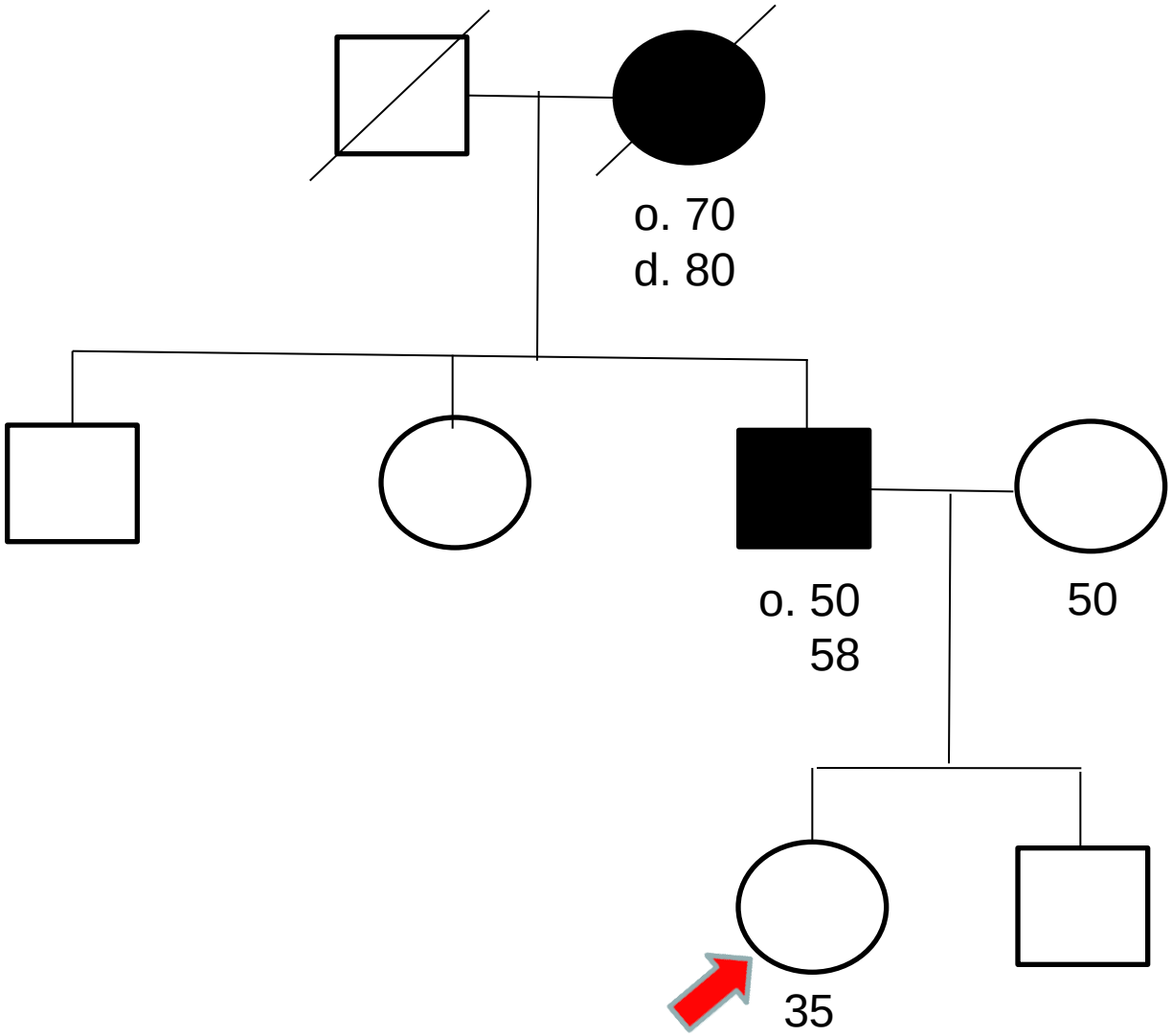
Focus: Case 13

- Commonly used and abused genetic terms: Penetrance and variable expressivity.
- Testing strategy for at-risk relatives: The specific mutation in an affected family member must be identified before relatives who might be affected can be tested.

Case Vignette 14

35 yo woman seeks genetic testing for Alzheimer Disease

- 58 yo father affected, onset 50yo
- Paternal Grandmother affected, onset 70yo, died age 80


o. 70
d. 80
o. 50
58
50
35

Case 14: Question

Is genetic testing indicated?

1. Yes
2. No
3. Maybe

Case 14: Question

Is genetic testing indicated?

1. Yes
2. No
3. Maybe

Case 14: Question

What tests should be done?

1. *APP* (amyloid gene)
2. *PSEN1* (presenilin 1)
3. *PSEN2* (presenilin 2)
4. *APOE*
5. DNA Banking

Case 14: Question

What tests should be done?

Discuss with family and agree on strategy.

Case 14: Question

Who should be tested?

1. Father
2. Daughter

Case 14: Question

Who should be tested?

1. Father
2. Daughter

Case 14: Question

No mutation identified in *APP*, *PSEN1* or *PSEN2*.

APOE genotype is ε4/ε4.

Would you do *APOE* testing on the daughter?

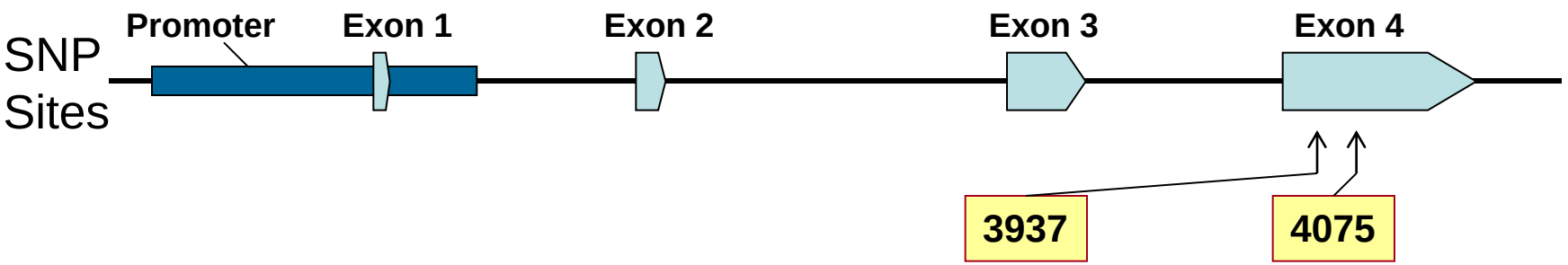
1. Yes
2. No
3. Maybe

Case 14: Question

Would you do *APOE* testing on the daughter?

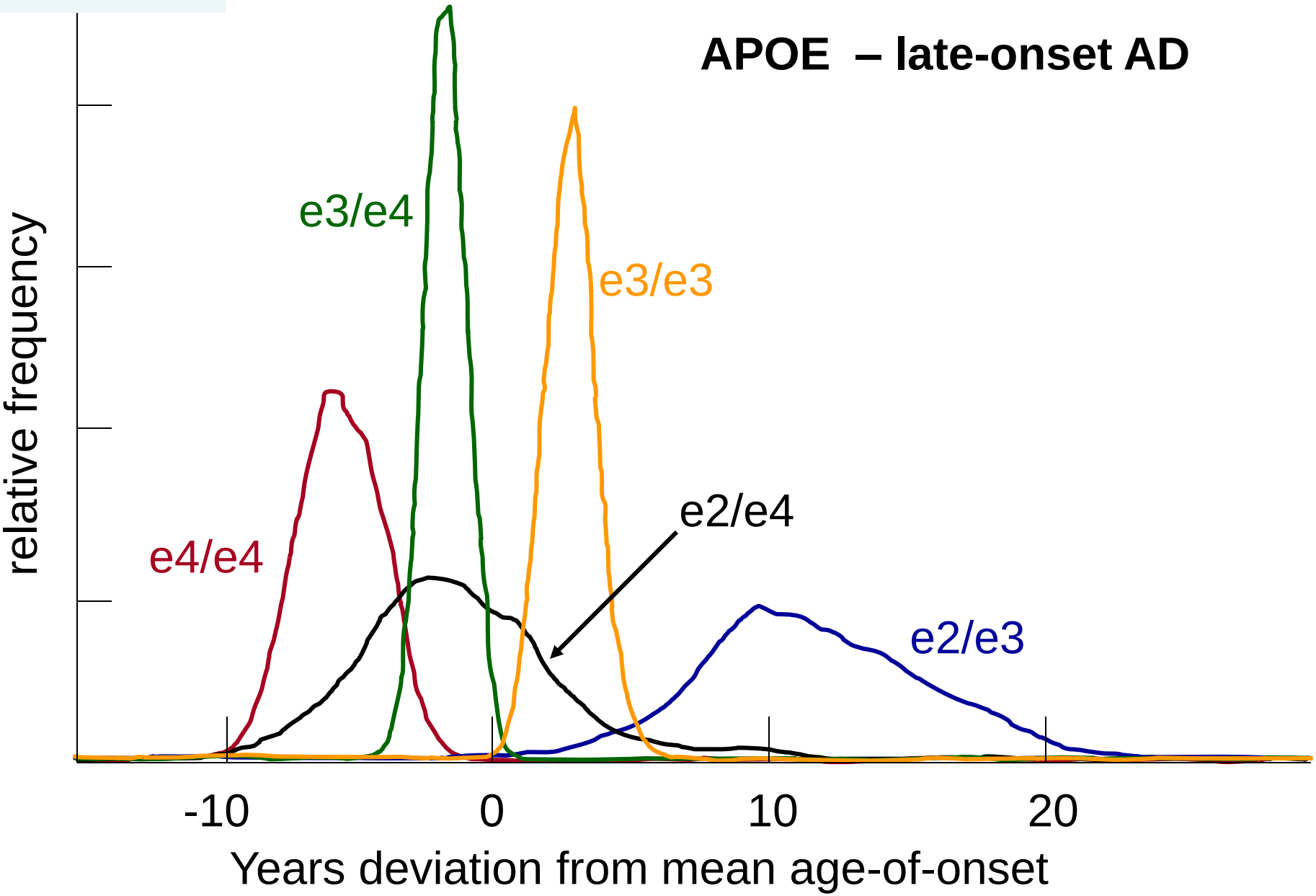
1. Yes
2. No
3. Maybe

APOE



Controls	AD		3937	4075
0.135	0.355	ε4	C (Arg)	C (Arg)
0.765	0.607	ε3	T (Cys)	C (Arg)
0.10	0.038	ε2	T (Cys)	T (Cys)

APOE


APOE – late-onset AD
relative frequency
e3/e4
e3/e3
e2/e4
e4/e4
e2/e3
-10
0
10
20
Years deviation from mean age-of-onset

REVEAL

- Boston University (Robert Green)
- Reveal Apo E Genotype
- Parent with AD
- Risk to develop AD
  - Knowing Apo E
  - Not knowing Apo E
- Assess impact on lifestyle/attitudes/behavior
- Role of genetic counseling
- Model for “new genetic medicine”

Case 14: Question

How would this situation be different if the father was deceased?

Focus: Case 14

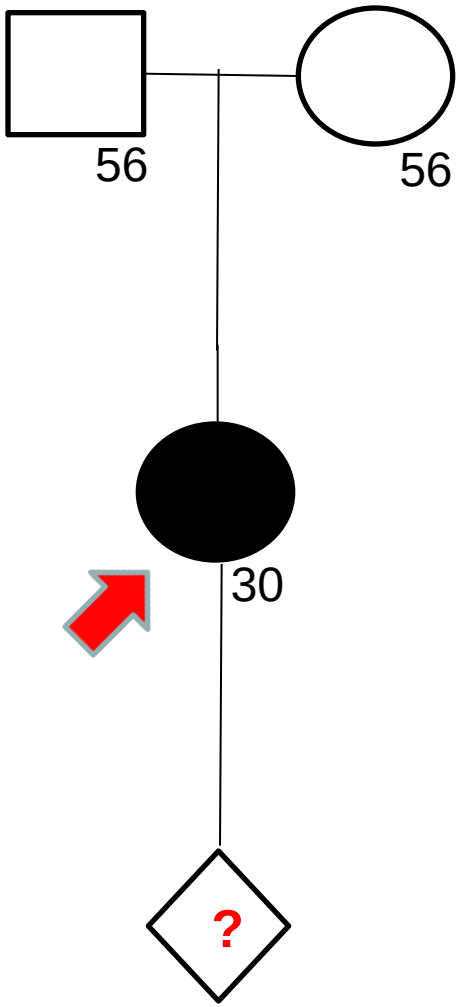
- Mendelian single gene causes vs. polygenic “risk” genes

- DNA banking

Case Vignette 15

A 30yo woman with a recent diagnosis of myotonic dystrophy is 10 weeks pregnant.

- Facial weakness
- Grip/percussion myotonia
- Myotonic discharges on EMG
- She has not had genetic testing
- Wants to know risk to fetus


56
56
30
?

Case 15: Question

Who do you test?

1. Mother
2. Fetus
3. Both
4. Neither

Case 15: Question

Who do you test?

1. Mother
2. Fetus
3. Both
4. Neither

Case 15: Test Result

- This woman has 450 CTG repeats in the *DMPK* gene associated with Myotonic Dystrophy (DM1).

Case 15: Question

Her fetus is at risk to develop:

1. Classic (Typical) Myotonic Dystrophy (DM1)
2. Congenital Myotonic Dystrophy (DM1)
3. Either 1 or 2

Case 15: Question

Her fetus is at risk to develop:

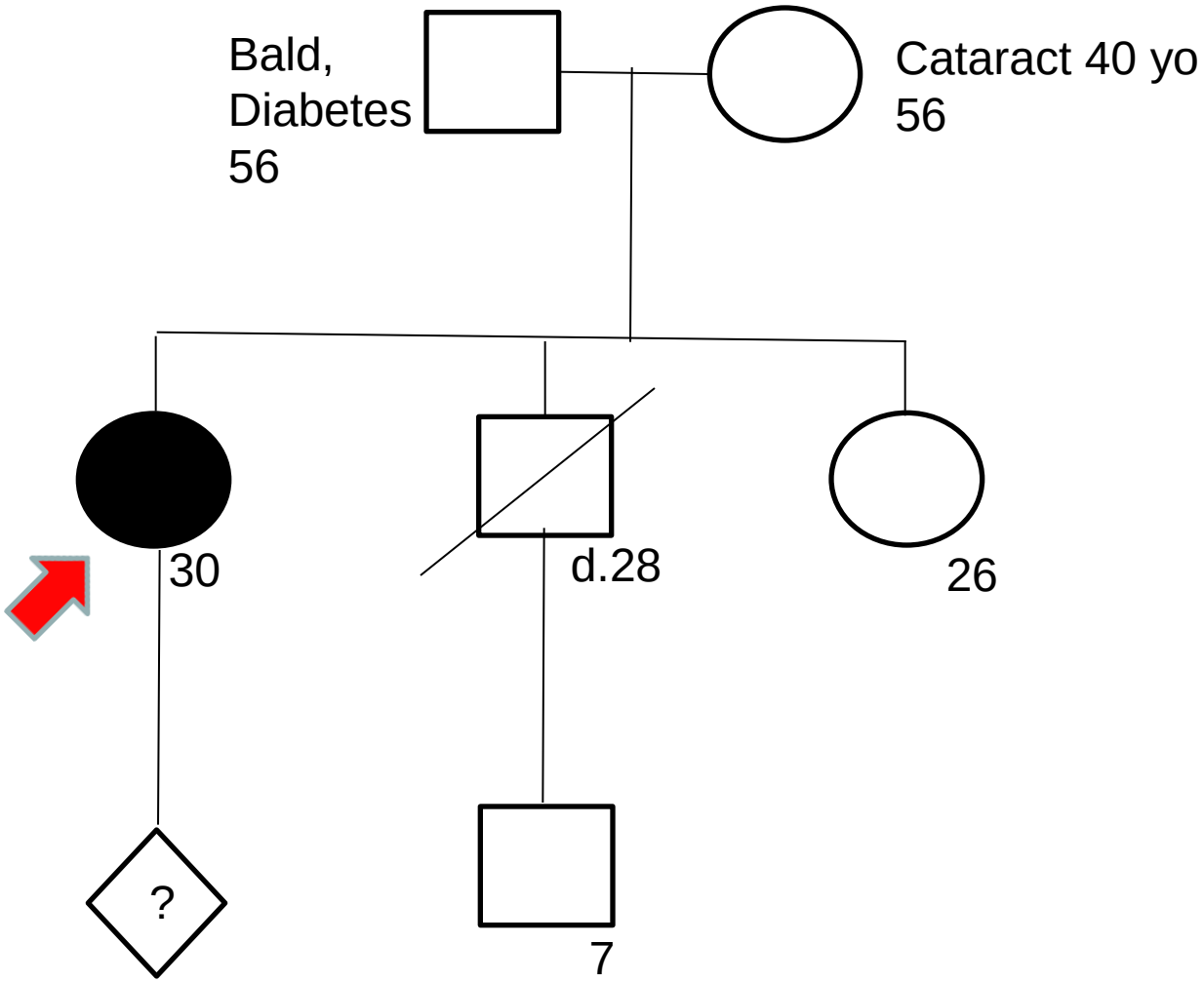
1. Classic (Typical) Myotonic Dystrophy (DM1)

2. Congenital Myotonic Dystrophy (DM1)

3. Either 1 or 2

Case 15: Question

- Her brother died suddenly at age 28.
- Her father has frontal baldness and diabetes.
- Her mother had cataract surgery at age 40.


Bald,
Diabetes
56
Cataract 40 yo
56
30
d.28
26
?
7

Case 15: Question

- To whom else in this family would you often genetic testing?

1. Mother
2. Father
3. Sister
4. All of the above

Case 15: Question

- To whom else in this family would you often genetic testing?

1. Mother
2. Father
3. Sister
4. All of the above

Case 15: Question

Would you test the 7yo nephew?

1. Yes
2. No
3. Maybe

Case 15: Question

Would you test the 7yo nephew?

1. Yes
2. No
3. Maybe

Focus: Case 15

- Genetic testing during pregnancy
- Testing children

Case Vignette 16

- 40 yo woman has CMT syndrome
- Neurologist orders full CMT panel (17 genes)
- Entire Panel negative
- Cost $15,000 (paid by insurance)

Case Vignette 16

- 16 yo son also develops CMT syndrome similar to mother's
- Would you order any CMT genetic tests?

1. Yes
2. No
3. Maybe

Case Vignette 16

Would you order any CMT genetic tests?

1. Yes
2. No
3. Maybe

Case Vignette 16

- Neurologist orders full CMT panel
- Entire Panel negative
- Cost $15,000 (paid by insurance)

Case Vignette 16

ERROR?

- Total Cost: $30,000
- What is the likelihood son has different genetic disease?

Focus: Case 16

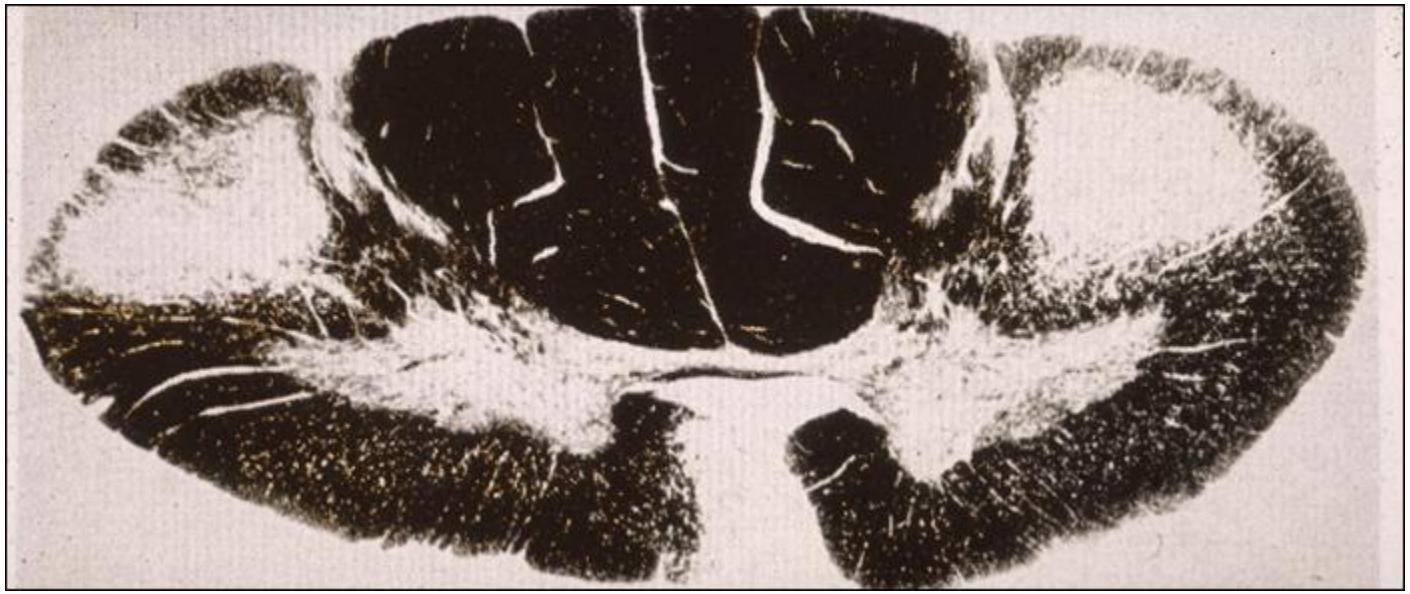
Duplicative testing in the same family

Case Vignette 17

A 38 year old woman has a family history of ALS.

- Her father, paternal aunt, and paternal grandfather have all died from ALS.
- Her 40 year old sister has ALS.
- The affected sister did not have an *SOD1* mutation.

ALS



Upper/Lower Motor Neurons

Case 17: Question

Does this family have familial ALS (FALS)?

1. Yes
2. No

Case 17: Question

Does this family have familial ALS (FALS)?

1. Yes

2. No

Case 17: Question

What proportion of ALS is familial?

1. 2%
2. 10%
3. 25%
4. 50%

Case 17: Question

What proportion of ALS is familial?

1. 2%
2. 10%
3. 25%
4. 50%

Amyotrophic Lateral Sclerosis Overview

Causes

Heritable Causes

An estimated 10% of individuals with ALS have at least one other affected family member and are said to have familial ALS (FALS).

Familial ALS can be categorized by mode of inheritance and subcategorized by specific gene or chromosomal locus.

Case 17: Question

What proportion of familial ALS can be attributed to *SOD1* mutations?

1. 5%
2. 20%
3. 50%
4. 90%

Case 17: Question

What proportion of familial ALS can be attributed to *SOD1* mutations?

1. 5%
2. 20%
3. 50%
4. 90%

Case 17: Question

What is the proband's risk for ALS?

1. 50 %
2. 25 %
3. 10 %
4. Background (population) risk
5. Unknown but higher than background risk

Case 17: Question

What is the proband's risk for ALS?

1. 50 %
2. 25 %
3. 10 %
4. Background (population) risk
5. Unknown but higher than background risk

Case 17: Question

- Should other genes be tested?

1. Yes
2. No
3. Maybe

Case 17: Question

- Should other genes be tested?

1. Yes
2. No
3. Maybe

Table 1. Molecular Genetics of Autosomal Dominant ALS

% Familial ALS	Locus Name	Gene
20%	ALS1	*SOD1*
2-10%	ALS4	*SETX*
	ALS6	FUS
	ALS8	*VAPB*
	ALS9	*ANG*
	ALS10	*TARDBP*
	ALS	*FIG4*
70-75%	Unknown	

Focus: Case 17

- Failure to detect a causative mutation does not exclude a genetic cause

- Tests for very rare disease are available and need to be individually considered